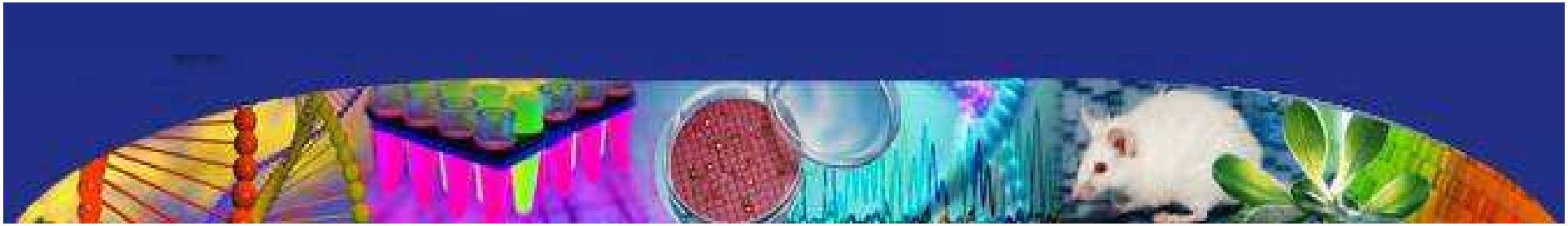
Updates to the *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)*

Jacqueline Corrigan-Curay, J.D. M.D.
Acting Director
Office of Biotechnology Activities
National Institute of Health



Disclosure

- No financial relationships to disclose.

Outline

- **Impetus for review of the *NIH Guidelines***
- **Process to date**
- **Outstanding Questions**
- **Next Steps**

National Science Advisory Board for Biosecurity

- **Advises the Secretary of the Department of Health and Human Services, the NIH Director and the heads of 15 departments and agencies with a role/interest in life sciences research**
- **Charged with recommending strategies for mitigating the potential for misuse of dual use biological research**
  - **Consider both national security concerns and the needs of the research community**

NATIONAL
SCIENCE
ADVISORY
BOARD FOR
BIOSECURITY

ADDRESSING BIOSECURITY CONCERNS RELATED TO THE SYNTHESIS OF SELECT AGENTS

DECEMBER 2006

NSABB Findings

- **Some practitioners of synthetic genomics/biology are:**
  - **Educated in disciplines that do not routinely entail formal training in biosafety; and**
  - **Uncertain about when to consult an Institutional Biosafety Committee (IBC).**
- **There is a need for biosafety principles and practices applicable to synthetic genomics/biology.**

Implementation of NSABB Recommendations

- **NSABB recommendations were considered through a trans-federal policy coordination process**
  - **Led by the White House Homeland Security Council and Office of Science and Technology Policy**
- **Recommendation on need for biosafety guidance accepted by USG with understanding that implementation would be through modification of the *NIH Guidelines* as appropriate**

Current Biosafety Guidance

- ***NIH Guidelines* are limited to synthetic DNA joined by recombinant methods**
  - **Does not cover synthetic DNA that is synthesized *de novo***
  - **Does not cover synthesized RNA viruses**
- **Biosafety in Microbiological and Biomedical Laboratories Manual (BMBL)**
  - **Agent specific, not technology driven**
  - **References *NIH Guidelines* with respect to synthetic recombinant molecules**

NIH Guidelines

Definition of Recombinant DNA

- **Molecules that are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or**
  - **Molecules that result from the replication of those described above**

Recombinant DNA Advisory Committee

- **Consider the application of the *NIH Guidelines* to synthetic biology**
  - **To what degree is this technology covered?**
  - **Does the scope need to be modified to capture synthetic biology research?**
- **Develop draft recommendations regarding principles and procedures for risk assessment and management of research involving synthetic biology**

Overall Approach

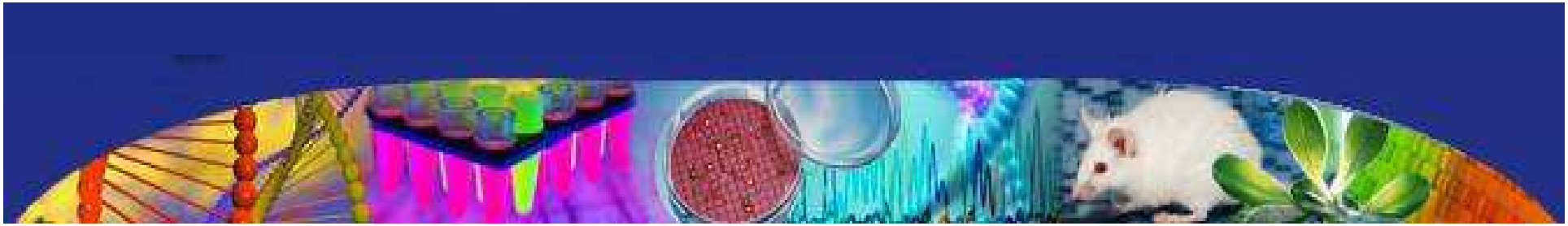
- **Capture the same products made by synthetic techniques that are currently covered under the *NIH Guidelines* for recombinant DNA research provided the same biosafety concerns are raised**
  - **Level of review based on risk not technique**
- **Develop a risk management framework that is based on the current science and what appears to be feasible in the foreseeable future**
- **Recognize that all not all future scientific developments can be anticipated, so that the *NIH Guidelines* will need periodic review and updating**

Section I-B. Definition of Recombinant and Synthetic DNA

(i) Recombinant nucleic acid molecules that are constructed by joining nucleic acid molecules and that can replicate in a living cell,

(ii) Synthetic nucleic acid molecules that are chemically or by other means synthesized or amplified, including those that are chemically or otherwise modified but can base pair with naturally occurring nucleic acid molecules, or

(iii) molecules that result from the replication of those described in (i) or (ii) above.



Replication: a Unique Risk?

- **The ability to replicate is one of the unique risks of recombinant DNA molecules**
  - **Potential ability to propagate in the lab, in exposed laboratory workers, and the environment**
- **Are the risks of non-replicating synthetic molecules comparable to rDNA?**
  - **For basic research**
  - **For clinical research**

Non-replicating Synthetic NAs: Basic Research

- **Exposure in the lab to a low dose of non-replicating synthetic nucleic acid sequence is considered low risk**
  - **Limited because even if the NAs enter a cell they cannot replicate and spread**
  - **Could not spread in the environment if released**
  - **Exposure similar to that of a chemical exposure; however nucleic acids are not toxic in and of themselves**

Basic Research with Synthetic NA

Proposed Exemption for those Synthetic Nucleic acids that:

a) can neither replicate nor generate nucleic acids that can replicate in a living cell, and

b) are not designed to integrate into DNA, and

c) do not produce a toxin that is lethal for vertebrates at an LD50 < 100 nanograms/kg, and

d) are not deliberately transferred into one or more human research participants (see Section III-C and Appendix M).

Human Gene Transfer

- **Doses used in human gene transfer considerably higher compared to that anticipated for inadvertent lab exposure**
- **Many human gene transfer trials use replication incompetent vectors; however, safety risks due to transgene effects, insertional mutagenesis, and immunological responses are independent of the replicative nature of the vector**
- **Human gene transfer often raises unique scientific, medical and ethical issues that warrant transparent oversight**

Non-Vector vs Vector Constructs

- **Comments agreed that vector constructs are human gene transfer, whether made synthetically or by recombinant means**

- **Considerable debate by RAC as to whether to include synthetic RNA and DNA not delivered by a traditional viral or bacterial vector**

Public Comments

- **Urged RAC and OBA to focus on distinctions between synthetic RNA and DNA oligonucleotide agents that are not delivered by vectors, including:**
  - **short half-life with more predictable pharmacokinetics**
  - **lack of ability to integrate into the genome**
  - **lack of replication or potential for inadvertent replication due to mobilization or complementation**
  - **lack of a transgene for coding a protein**

Spectrum of Biologic Therapies

Long-Term Replacement of Function of Defective Gene (RV-ADA gene for ADA-SCID)

Designing Tumor Specific T cells (Chimeric Antigen Receptors for cancer)

Regulation of Gene Expression (plasmid zinc finger-transcription factor to upregulate VEGF-A for PAD)

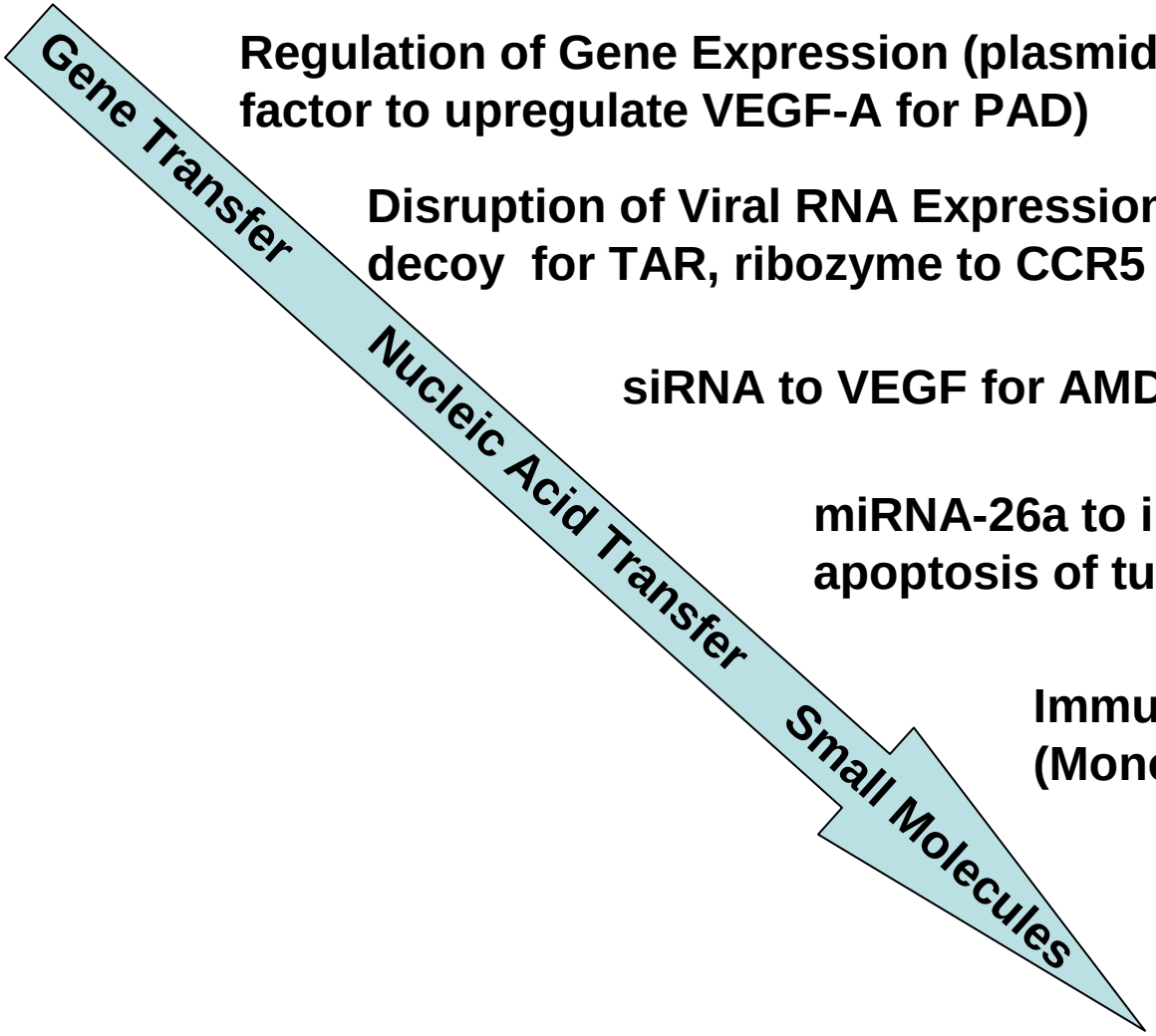
Disruption of Viral RNA Expression (LV-shRNA to tat/rev,RNA decoy for TAR, ribozyme to CCR5 for HIV)

siRNA to VEGF for AMD

miRNA-26a to inhibit Cyclin D2/E2 for apoptosis of tumor cells

Immunotherapeutic drugs (Monoclonal Antibodies, Gleevec)

Recombinant Proteins (Insulin)



DNA Oligonucleotides

- Mature Field
- Mechanisms of action well characterized

RNA Oligonucleotides

- **Relatively new field, clinical trials ongoing but rapidly expanding applications**
- **siRNA and miRNA have pleiotropic effects that are conserved across species**
  - **Individual miRNAs have been shown to suppress the production of hundreds of proteins (Baek, D., *et. al*., 2008; *Nature* 455, 64)**

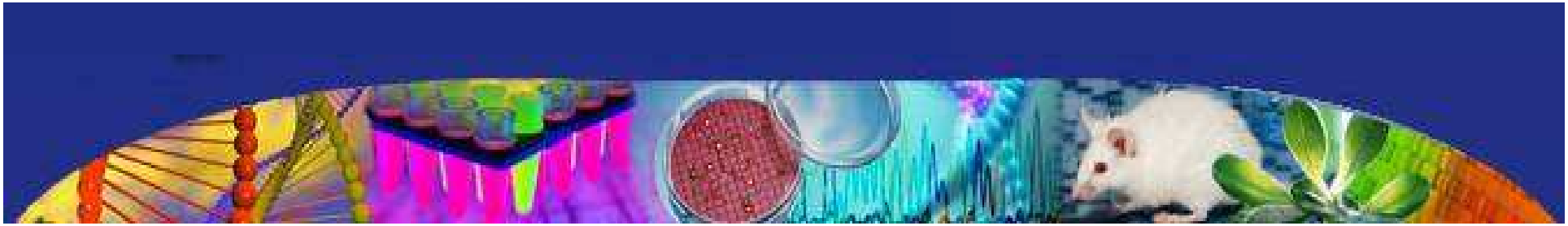
Are these potential safety concerns?

- **In preclinical models:**
  - **Efficacy of RNAi for macular degeneration was result of immune stimulation of toll-like receptors (Kleinman, M., *et. al*., 2008; *Nature* 452:7187).**
  - **Administration of shRNAs led to a fatal toxicity due to unanticipated competition with endogenous miRNA processing (Grimm, D. *et. al.*, 2008; *Nature* 441: 537).**
  - **Administration of siRNA targeting macrophage migration inhibitory factor (MIF), a cytokine with well described roles in cell proliferation, tumorigenesis, and angiogenesis, unexpectedly led to enhanced proliferation of breast cancer cells rather than the expected apoptosis (Armstrong, M. *et al*., 2008; *J. Immunology* 180:7125).**

Could epigenetic changes have long term consequences ?

- **Can siRNA and miRNA lead to long-term gene silencing (Hawkins, P., *et. al.*, 2009 *Nucleic Acid Res*. 37(9):2984; Kim, D. *et. al*. 2009; *PNAS* 105:16230).**

- **What are the clinical implications?**



NEWS & ANALYSIS

RNA-BASED THERAPIES | MARKET INDICATO

Table 1 | Comparison of gene-silencing drug strategies

Characteristic	Old generation antisense	Ribozyme
Molecular structure	ssRNA or ssDNA	RNA molec… enzym…
Catalytic mechanism of action	No	
Activity dependent on RNA structure	Yes	
Specificity / therapeutic doses		
Stability		
Potency		
Delivery options		

Source: scientific litera…

LIFE SCIENCE TECHNOLOGIES

RNAi therapeutics

RNAi THERAPEUTICS:

A TWO-YEAR UPDATE

"I think they are potentially superdrugs."

Table 2 | **Selected RNA-based therapies in development**

Company	Programme	Indication	Status
Antisense			
Isis	ISIS301012	High cholesterol	Phase II
	ISIS113715	Diabetes	Phase II
OncoGenex, Isis	OGX-011	Cancer	Phase II
Eli Lilly, Isis	LY2181308	Cancer	Phase I
AVI BioPharma	Resten	Restenosis	Phase II
	AVI-5126	CABG	Phase I/II
	AVI-4065	Hepatitis C	Phase II
Topigen	TPI-ASM8	Asthma	Phase I
Lorus Therapeutics	GTI-2040	Renal cell carcinoma	Phase II
Aptamer			
Archemix	ARC1779	Acute coronary syndrome, percutaneous coronary intervention	Phase I
Antisoma, Archemix	AS 1411	Renal cancer, acute myeloid leukaemia	Phase II
Small-interfering RNA			
Opko Health	Bevasiranib (C and 5)	Wet AMD	Phase III
Allergan	AGN211745 (Sirna-027)	Wet AMD	Phase II
Silence Therapeutics, Quark Biotech, Pfizer	RTP801i	Wet AMD	Phase I
Alnylam	ALN-RSV01	RSV infections	Phase II

AMD, age-related macular degeneration; RSV, respiratory syncytial virus.

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TECHNOLOGY FEATURE RNA INTERFERENCE

Homing in on delivery

The scientific community now seems convinced that small RNAs will become therapies, if new tools can help these large molecules to make it safely into cells. Monya Baker reports.

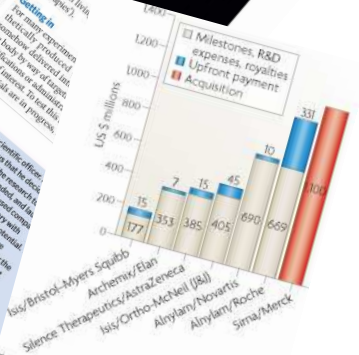

Figure 1 | **Selected RNA therapeutics biotechnology and pharmaceutical company deals.**

Weighing the Evidence

Clinical Trials without Evidence of unexpected toxicity Or long-term adverse effects

Emerging Basic Research highlighting the potential limits of our understanding

Next Steps

- **Revisit the data at the June 17, 2010 Meeting of the Recombinant DNA Advisory Committee, Bethesda MD**

- **Webcast:**
  http://oba.od.nih.gov/rdna_rac/rac_meetings.html