

Biocontainment for Research with Partial Genomes of Viruses in Tissue Culture

under the

NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines)

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Outline

- **Review of Section III-E-1: Biocontainment for Research with Partial Genomes of Eukaryotic Viruses in Tissue Culture.**
- **Impetus for Review and Proposed Amendments.**
- **Evolution of Proposed Changes.**
- **Revised Proposal and Outstanding Issues for Discussion.**

Section III-E-1 Current Requirements

- Section III-E-1 allows investigators to initiate research with partial viral genomes in tissue culture at Biosafety Level (BL) 1 containment upon registration of their experiment with the Institutional Biosafety Committee (IBC).
- To work under this section the virus must contain less than two-thirds of the genome from any family of viruses and there must be no helper virus present.
- This section is designed to facilitate initiation of low-risk research.
- The IBC is nonetheless required to review the research.

Impetus for Proposed Changes

- **RAC review of research with synthetic nucleic acids and biosafety led to discussions of Section III-E-1**
 - **A question arose as to whether synthetic techniques might be used to generate a functional virus containing less than two-thirds of the genome.**
 - **There was recognition that rescue of a replication competent virus could occur in the absence of helper virus through other mechanisms, and in particular, via helper function.**

March 2009 Proposed Revisions

- In March 2009, OBA published a proposal in the Federal Register (74 FR 9411) to amend Section III-E-1 by:
 - Changing the criterion regarding the size of the virus genome deletion:
 - From two-thirds to **ONE-HALF**
 - Requiring the PI to provide evidence that the resulting nucleic acids in the tissue culture cells are not capable of producing a replication competent virus in addition to demonstrating the absence of any helper virus.

Public Comments – March 2009 Proposal

- Public comment on this proposal raised two issues:
 - There is research that has been safely conducted for many years under this section with viruses that contain more than one-half of the genome but less than two-thirds: e.g., viral replicon particles of Venezuelan Equine Encephalomyelitis.
 - Rather than a quantitative standard based on deletion size, does the current understanding of virus biology allow for a reduction in containment based on a functional impairment?

Revised Proposal – April 2010

- After consultation with the RAC, OBA proposed additional amendments:
 - Retain the proposed criterion that one could work under this section if only one-half of the genome was present.
 - Clarified that this only applied to Risk Group (RG) 3 and 4 viruses because research with less than one-half of the genome of a RG2 virus is already exempt from the *NIH Guidelines*.

Revised Proposal – April 2010 (cont'd)

- **Include functional criteria that would allow reduction of containment to be based on the removal of one or more viral genes that are essential for cell-to-cell transmission.**
 - **Removal of such genes should prevent the propagation of virus and its ability to cause disease.**
- **Clarified that containment for research with retroviruses and lentiviruses that have the potential to transduce human cells should not be less than BL2.**

Section III-E-1 – Proposal

Published in 75 FR 21008 - April 22, 2010

An investigator can initiate work at BL1 containment in tissue culture upon notification of the IBC if:

- 1) No more than half of the eukaryotic viral genome is present
- OR**
- 2) There is a complete deletion in one or more essential viral capsid, envelope, or polymerase genes required for cell-to-cell transmission of viral nucleic acids and the investigator provides the IBC with evidence such as sequence or other appropriate data to demonstrate that there is a complete deletion of genetic sequence such that these functions can not be rescued through homologous recombination.

Section III-E-1 – Proposal (cont'd)

Published in 75 FR 21008 - April 22, 2010

AND

In both situations there must be evidence that:

1. The resulting nucleic acids are not capable of producing a replication competent virus in a cell line that would normally support replication of the wild-type virus
2. There is no helper virus present

Notwithstanding above, a minimum of BL2 containment is required for experiments with retroviruses and lentiviruses that have the potential to transduce human cells and cause insertional mutagenesis.

Section III-E-1

Additional Biosafety Concerns -- June 2011

- Review of research to create a defective RG4 agent raised concerns about the advisability of working with these viruses under BL1 containment prior to review.
 - The possibility of a rare event resulting from homologous and/or non-homologous recombination could result in the rescue of a potentially lethal virus at lower containment.
 - Documenting that rescue of replication competent virus would not occur, presents considerable challenges including:
 - Establishing the sensitivity of biological and physical detection methods for replication competent virus.
 - Designing a statistically rigorous experiment that could demonstrate the strength of negative data given the likelihood of low incidence rates associated with such events.

Section III-E-1 – Proposed Revision

June 2011

- **Exclude Risk Group 4 Viruses from Section III-E-1**
 - Given the potentially serious consequences associated with an automatic lowering of containment for RG4 viruses, it is prudent to have such high-risk research reviewed prior to initiation.
 - The impact of this proposed change on the research community should be minimal.

Regarding RG2 and RG3 Agents

Do the same risks identified for RG4 agents apply equally to all RG3 and RG2 agents?

Can some or all RG3 and RG2 viruses be genetically altered to guarantee a sufficient degree of safety that would allow the automatic lowering of containment to BL1 concurrent with IBC registration as stated under Section III-E-1.

- RG3 viral agents include a majority of RNA viruses and fewer DNA viruses**
- RG2 viral agents include a large number of both DNA and RNA viruses**

Biosafety Considerations for RG2 and RG3 Agents

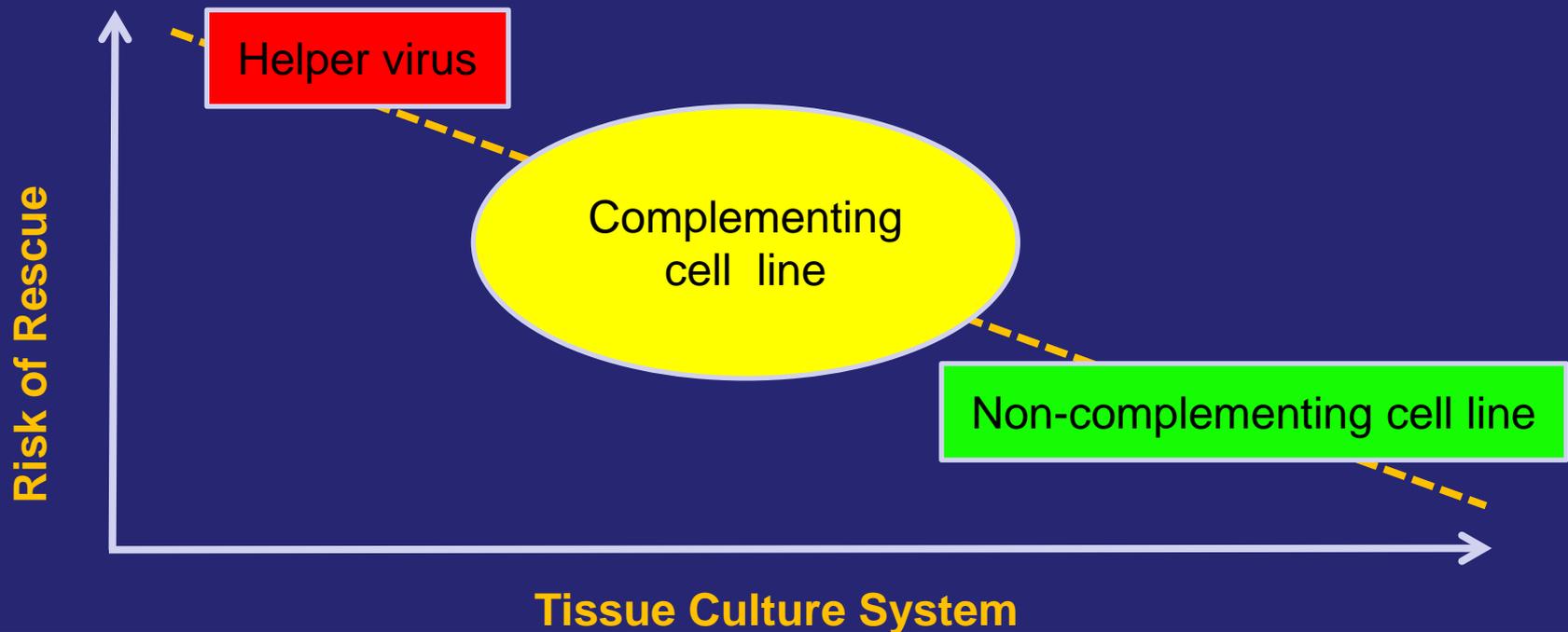
As recombination events are the focus of the biosafety concern, the likelihood of these events is dependent in part:

- On the nature of the virus, for example:
 - Negative single-strand (nss) RNA viruses may be rescued by non-homologous recombination events. These are known to be to be very rare occurrences.
 - Positive strand and segmented RNA viruses can be rescued by both homologous and non-homologous recombination. This is also a relatively rare occurrence.
 - DNA viruses, many of which are RG2 viruses, have a much higher likelihood of rescue by recombination events as compared to nssRNA viruses.

And...

Biosafety Considerations for RG2 and RG3 Agents (cont'd)

- On the physiological state of the virus, for example:
 - Rescue events are dependent on viral (or cellular) replication and are more likely to occur in the presence of helper virus, to a lesser extent in complementing cells, and to the least extent in non-complementing cells.



Potential Approaches

- **Allow only a step down approach to containment**
 - **Defective RG2 viral agents are contained at BL1**
 - **Defective RG3 viral agents are contained at BL2**
 - **Requiring BL2 containment is not consistent with a Section III-E experiment in which initiation of an experiment simultaneous with IBC registration is based on a presumption of safety that allows biocontainment to be BL1.**

Potential Approaches

- **What kind and how much evidence will be required to lower containment?**
 - **The data necessary to demonstrate that a tissue culture system will not support a rescue event will be dependent on several factors:**
 - **The nature of the virus, the likelihood of recombination and the cell line in use (i.e. complementing or not)**
 - **The challenge is to provide consistent guidance to PIs regarding what data need to be provided to support lowering of containment as a function of the risks associated with the tissue culture system used.**

Conclusions

Research with defective RG4 viruses (based on deletions of one or more genes) should be reviewed by the IBC prior to initiation.

Further discussion is needed to revise the criteria for RG2 and RG3 virus research that should be subject to Section III-E-1 (i.e. lowering containment to BL1 prior to IBC review).

- **Articulate guidance for investigators regarding the data needed to lower containment with defective RG2 and RG3 viruses in light of the differences in risks of recombination among different viruses and the cell lines being used propagate and maintain them.**