Guidance for Work with Adenovirus and Adeno-associated Virus and Viral Vectors

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I. Introduction

Adenoviruses (AV) are a group of common non-enveloped viruses that can infect cells by aerosol, droplet exposure, ingestion or injection. They can infect a broad range of tissues, causing mostly benign infections such as nasal congestion, runny nose, cough, and conjunctivitis. Infection may also affect the gastrointestinal tract. AVs have a linear double stranded DNA genome and are able to replicate in the nucleus of vertebrate cells using the host’s replication machinery. AV vectors have been engineered to carry exogenous DNA into hosts cells. These genetic modifications can include removal of essential genes which render the vector replication incompetent. Their use in gene therapy takes advantage of the virus’s inherent ability to infect and transduce cells to produce the desired therapeutic or immunized outcome. They are able to encode proteins without integrating into the host cell’s genome. However, exposure to AV vectors can induce varied immunological responses in the host, depending on the serotype.

Adeno-associated viruses (AAV) are non-enveloped viruses considered to be non-pathogenic and not currently known to cause disease in humans even though the virus will integrate in the host genome. AAVs have a linear single-stranded DNA genome. They are called adeno-associated viruses because they are often found in cells that are simultaneously infected with adenovirus. AAVs have been used as viral vectors due to their efficient gene transfer, transient or stable transgene expression, and lack of induction of strong immune responses. Production of AAVs requires a helper plasmid, usually in the form of a helper Adenovirus, although Herpes Simplex Virus has also been used successfully. A primary safety concern is the presence (contamination) of helper virus when growing these vectors. Also, if no helper virus is present, AAV can insert DNA stably into the host genome, usually at a specific site on Chromosome 19.
Recombinant vectors lose this specificity found in wild type AAVs and appear to randomly integrate, posing a theoretical risk of insertional mutagenesis.

II. Applicability

This document applies to all employees of GSU including faculty, staff, student workers, visiting researchers, adjunct faculty, and other GSU affiliates performing tasks which may come into contact with adenovirus, adenoviral vectors, adeno-associated virus, adeno-associated viral vectors, or biological materials harboring these agents.

III. Background

Adenovirus/Adenoviral Vectors
Replication-competent (wildtype) and replication-deficient AVs are classified as Risk Group 2 agents (agents that are associated with human disease, which is rarely serious and for which preventive or therapeutic interventions are often available). According to the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules 2019 (NIH Guidelines), Section III-D-3: “Experiments Involving the Use of Infectious DNA or RNA Viruses or Defective DNA or RNA Viruses in the Presence of Helper Virus in Tissue Culture Systems,”
applies when helper virus is used and requires IBC approval prior to initiation of experiments. However, Section III-E-1 applies when experiments involving the formation of recombinant or synthetic molecules containing no more than 2/3 of the genome of any eukaryotic virus are used, is considered replication defective, and used in the absence of helper virus. These experiments may be exempt under the NIH Guidelines, but they still require administrative or expedited review by the Biosafety Office and/or IBC.

**Adeno-associated Virus/Adeno-associated Viral Vectors**

Appendix B-1 of the NIH Guidelines assesses replication-competent (wildtype) and replication-deficient AAVs as Risk Group 1 (agents that are not associated with disease in healthy adult humans). Typically, Section III-E-1 will apply as long as the transgene does not encode either a potentially tumorigenic gene or a toxin molecule and are produced in the absence of a helper virus.

### IV. Considerations

The Biosafety Office reviews protocols involving biological materials, genetic components, and animals/animal products with emphasis on the health and safety of the researchers, the animal care staff, the community, and the environment. Reviews involve communication with the laboratory staff and the Principal Investigator to properly identify procedures and materials which will impact the risk assessment process. The risk assessment is then completed in conjunction with the GSU Research Occupational Health Nurse Practitioner and the Occupational Health Officer. The IBC and IACUC are involved when protocols involve the use non-exempt nucleic acid molecules and/or animals, respectively. The creation and/or use of adenoviral and adeno-associated viral vectors fall under the definition of recombinant nucleic acid molecule research as outlined in the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, experiments must be submitted for review and approval by the IBC prior to initiation.

A. Biocontainment

Utilizing the hierarchy of controls, elimination, or substitution of the hazard, if possible, should be considered. Unfortunately, it is difficult to eliminate the need to use viral vector systems; but we can substitute the use of wild-type virus and first-generation viral vector systems with later generation systems if it does not impact research goals.

**Adenovirus/Adenoviral Vectors**

- First generation adenoviral vectors are stripped of the regulatory genes E1 and E3 which are necessary for replication. However, they can replicate in E1-containing mammalian cells such as HEK293 cells. Importantly, recombination-competent adenovirus (RCA) can be generated if the E1 gene from the packaging cell line is transferred into the adenoviral vector by recombination. The probability of producing replication competent adenovirus (RCA), although low, increases with each successive amplification. It is suggested to use early amplification stocks when needed to produce additional quantities of adenovirus and test for the presence of RCA in the viral stock by doing a plaque assay. The cloning capacity of first generation adenovirus is limited to 8.2 kb.

- Second generation adenoviral vectors were designed to have increased cloning capacity, decreased potential for the generation of RCA and reduced immunogenicity. Along with the E1 and E3 genes, non-structural genes E2 and E4 are also deleted in 2nd generation adenoviral vector, thereby increasing cloning capacity to ~12 kb.
• Third generation adenoviral vectors are devoid of all viral coding sequences and retain only the inverted terminal repeats (ITR) and packaging signal in cis. Therefore, co-infection with a helper adenovirus is required to provide the viral proteins in trans. These adenoviral vectors have generated high interest for gene therapy due their increased cloning capacity of up to 36 kb.

Experiments using first generation AV vectors may require additional precautions when produced in mammalian cell lines containing E1. Only one recombination event required to generate replication competent virus.

Adeno-associated Virus/Adeno-associated Viral Vectors
• There are currently 12 different AAV serotypes (AAV1-12) used as gene therapy vectors although there are several other variants known to exist.
• AAV serotypes 2, 3, 5, and 6 were discovered from human cells and AAV serotypes 1, 4, and 7-11 have non-human primate origins. AAV2 is commonly used for viral vector technology.
• The AAV genome has rep (replication) and cap (capsid) reading frames located between inverted terminal repeats (ITRs). Rep encodes four non-structural proteins necessary for replication: Rep78, Rep68, Rep52, and Rep40. Cap encodes three structural proteins: VP1, VP2, and VP3.
• A variety of viral vectors have been created lacking both rep and cap.
• AAV vectors are small, only 4.7 kb. This limitation has resulted in the use of strategies to increase insert size such as the use of dual vectors.

The results of the risk assessment will determine the laboratory biosafety level (BSL) and animal biosafety level (ABSL); which are a combination facilities, practices, and equipment necessary to reduce the risk to an acceptable level.

BSL-2 containment is often considered appropriate in the laboratory setting for research involving the use of AVs or AV vectors. The practices, equipment, and facilities required for BSL-2 containment are described in Appendix G-II-B-2 of the NIH Guidelines. Animals exposed to AVs or AV vectors should be housed in ABSL-2 conditions; lowering containment to ABSL-1 after initial delivery and infectivity period must be requested and approved by the IBC. BSL-1/ABSL-1 containment is typically appropriate for AAVs or AAV vectors. However, the IBC may require BSL-2/ABSL-2 containment when using AAV vectors containing transgenes expressing oncogenic protein or toxins or when generated using helper viruses of human origin.

Decisions about containment should consider a range of parameters/considerations including:
• the nature of the vector system and the potential for regeneration of replication competent virus from the vector components,
• the nature of the transgene insert (e.g., known oncogenes or genes with high oncogenic potential may merit special care)
• the vector titer and the total amount of vector,
• the inherent biological containment of the animal host, if relevant, (both biosafety issues with animal husbandry and housing after the initial injection AND the initial inoculation itself, will be assessed)
• negative replication competent adenovirus (RCA) testing, if needed.
Reducing the containment level from ABSL-2 to ABSL-1 involves the following procedures:

- Surgical infections are conducted and recipient animals subsequently housed at ABSL-2 for a minimum of 72 hours following infection.
- Specific ABSL-2 conditions are arranged with the director or designee of DAR and the veterinary staff at each research facility.
- In special cases, in arrangement with veterinary staff, specified ABSL-2 containment animal racks may be used within an otherwise ABSL-1 designated vivarium room.
- There will be specific signage / labeling on each ABSL-2 cage indicating the viral vector administered as well as the date of administration, and these cages will not be allowed out of the ABSL2 containment space.

B. On the fourth day following infection, animals can be transferred to ABSL-1 standard conditions by the DAR staff. The animals will be transferred to a clean cage, and the ABSL2 cage will stay in the ABSL-2 quarantine space for appropriate waste disposal and cleaning. Once animals have been transferred to ABSL-1, they can be used within ABSL-1 behavioral facilities, etc. as with other ABSL-1 animals. Medical Clearance
Personnel must be enrolled in the Research Occupational Health Program and adhere to the practices and procedures outlined in the Adenovirus/Adenoviral Vector and/or Adeno-associated Virus/Adeno-associated Vector Exposure Control Plans. There are no preventative measures (i.e., vaccinations) currently available for adenovirus or adeno-associated virus.

C. Training
Each institution conducting or sponsoring recombinant or synthetic nucleic acid molecule research which is covered by the NIH Guidelines is responsible for ensuring appropriate training in laboratory safety and implementation of the NIH Guidelines. Personnel must complete NIH Guidelines Training online every 3 years and Biosafety training online annually (initial, then refresher). The Principal Investigator is required to train laboratory personnel on safe handling procedures specific to agents and procedures associated with the laboratory’s project aims. The Division of Animal Resources will train on procedures related to animal handling and manipulation. Animal procedures described in an IACUC protocol will undergo post-approval monitoring (PAM) on an annual basis to ensure compliance with the approved protocol.

D. Safe Handling/Signage
Personnel are required to wear the PPE prescribed by the risk assessment and corresponding BSL/ABSL. This will generally consist of gloves, laboratory coat or gown, and eye protection when splash, splatter or spray is anticipated. All work should be performed in a biosafety cabinet, unless an exception is approved by the Biosafety Office. Entry doors where these materials will be used should have signage posted to notify nearby personnel of work with potentially infectious materials. All work surfaces should be decontaminated with a disinfectant suitable for the pathogens being used (i.e., 10% Bleach solution, prepared fresh daily or other approved disinfectant). Note: For AAVs, due to the stability of the protein capsid, these viruses are stable for up to a month at room temperature. Alcohol-based disinfectants are NOT effective. A broad-spectrum disinfectant, such as sodium hypochlorite (i.e. 1:10 bleach solution) must be used to inactivate AAVs.

E. Waste Disposal
Solid biohazardous waste potentially contaminated with these materials such as culture vials, plates, plastic tubes, animal bedding, animal carcasses, etc., should be autoclaved and/or packaged for incineration in a
biohazard box. Containers used to collect biohazardous wastes must be labeled with biohazard stickers and lids are to remain secured/closed when not in use. Liquid waste must be treated prior to disposal (i.e., bleach - final volume 10% for 15 minutes or autoclaving) down the sink with copious amounts of water. Sharps waste, such as broken glass, pasteur pipets, razor blades, and needles, must be disposed of into biohazard sharps containers. When the fill line on the sharps container is reached, the lid must be secured/closed. More information regarding sharps handling and disposal can be found in GSU’s Guidance for Safe Handling of Sharps. Biohazardous wastes can be scheduled for pickup by the Research and Environmental Safety Office via Chematix.

F. Emergency Response
- Inside of containment – Remove and replace any contaminated PPE. Gently cover spill with a dampened absorbent material such as a paper towel. Apply 10% bleach, starting at the perimeter of the spill and working towards the center. Allow for a 30-minute contact time. Clean up spill and repeat, if necessary. Follow up with a 70% Ethanol or another approved disinfectant. Place all waste clean-up in biohazardous waste container to be autoclaved.
- Outside of containment – Report incident to PI and Research and Environmental Safety (RES) 404-413-3540. Don appropriate PPE (clean laboratory coat/gown, safety glasses, and gloves). Gently cover spill with absorbent material such as a paper towel. Apply 10% bleach, starting at the perimeter of the spill and working towards the center. Allow for a 30-minute contact time. Clean up spill and repeat, if necessary. Follow up with a 70% Ethanol or another approved disinfectant. Place all waste clean-up in biohazardous waste container to be autoclaved.

G. Exposures
All exposures to recombinant or synthetic nucleic acid materials should be evaluated by a medical practitioner. Consult the GSU Critical Event Guide in the event of an exposure. The PI/supervisor and RES should be notified as soon as possible. Exposure symptoms differ depending on route of transmission. Most infections are mild and require no therapy or only symptomatic treatment. Because there is no virus-specific therapy, serious adenovirus illness can be managed only by treating symptoms and complications of the infection.

H. Reporting
Follow posted injury reporting procedures found on the Critical Event Guide and Insurance and Risk Management posters. The PI/supervisor and RES should be notified as soon as possible. Exposures or spills of recombinant or synthetic nucleic acid molecules may require reporting to the NIH.

VI. References
- https://www.cdc.gov/adenovirus/index.html
- https://en.wikipedia.org/wiki/Adenoviridae
- http://www.ehso.emory.edu/research-safety/bars/adenovirus.html
- https://thenativeantigencompany.com/repurposing-adenoviruses-as-vectors-for-vaccines/
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• https://ehs.uky.edu/docs/pdf/bio_viral_vectors_0001.pdf
• https://ehs.stanford.edu/reference/adenovirus-fact-sheet
• https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5482513/

Guidance Authorization

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