SAFE SCIENCE!

Safe Science Therapeutics, Inc., Institutional Biosafety Committee (IBC)
Meeting Agenda

Meeting Date: May 17, 2023
Time: 7:00 PM to 8:00 PM
Location: Virtual Meeting

1. Welcome & Introductions

2. Review and Approval of Minutes from the 2022 IBC meeting

3. Biosafety Officers Report

4. Member Training: Lentiviral Vectors

5. New Protocol Review: Protocol #23-02, "Therapeutic Potential of HDAC6-inhibitors in Lung Adenocarcinoma," Dr. H. Jones

6. Other topics or questions (if any)

SAFE SCIENCE!

Safe Science Therapeutics, Inc., Institutional Biosafety Committee (IBC)
Meeting Minutes

Meeting Date: May 18, 2022
Time: 6:00 PM to 8:00 PM
Location: Virtual Meeting

Start Time: 6:00 PM
End Time: 7: 45 PM

Present: Jessica Healey (Chair), Marissa Cardwell (Subject Matter Expert), Mayomi Omebeyinje (Subject Matter Expert), Eddie Hall (Community Member), Patrick Macdonald (Community Member), Dianna Olukotun (Biosafety Officer)

Guests: None

1. Welcome & Introductions –All IBC members

2. Review and Approval of Minutes from the 2021 IBC meeting

3. Overview of protocol amendment
    - Lentivirally-transduced human dendritic cells will be used in mice
    - Covered under Section III-D-4 of the NIH Guidelines
    - Covered under the OSHA BBP Standard
    - Work will be performed under Animal Biosafety Level 2 containment in the onsite vivarium

4. Discussion of protocol registration
    - Questions/Comments from Community Member:
        - Update NIH Guidelines citation to reflect new animal work
        - Will oncogenic inserts be used?
            - PI says that they will not.

        - What disinfectants will be used with for the human cell work? An EPA-registered disinfectant for HIV and HBV must be used.
            - 70% ethanol and Cavi-Wipes will be used.
            - Cavi-Wipes are EPA-registered for use against HIV and HBV
    - Questions/Comments from Community Member
        - There is a mention of the use of low doses of staph enterotoxins. More information is needed regarding quantities, doses, etc. to ensure that Select Agent requirements aren't being triggered. The toxins table on the form isn't filled out.
    - Required changes to the protocol:
        - Correct NIH Guidelines Citation (Biosafety Officer)
        - Provide clarifying information for disinfectant use (Biosafety Officer will follow up with Lab)
        - Provide clarifying information for toxin use (PI)

    Protocol is Conditionally Approved pending changes noted above (Administrative Review).

5. Other topics or questions (if any)
    - Eddie will be on vacation for most of July.
    - Unless there is a major change in the research, the next committee meeting will most likely be scheduled for sometime next Spring

6. Meeting Adjourned at 7:45

SAFE SCIENCE!

Safe Science Therapeutics, Inc.
123 Maine Street
Cambridge, MA 02139

Biological or rDNA Use Registration Form		
P.I. name, degree: H. Jones, PhD	Primary lab contact name: S. Round	
Title: Therapeutic Potential of HDAC6-inhibitors in Lung Adenocarcinoma	Email: RoundS@safescienceinc.com	
Phone: 617-123-4567	Phone: 774-867-5309	
Protocol Number: 23-02		
☒ New	☐ Amendment or Modification	☐ Renewal

LABORATORY PROTOCOL PROJECT DESCRIPTION

Describe the specific experiments, assays, and procedures that will be conducted. (10-20 sentences) If multiple experiments will be conducted, include sections with numbering and subtitles for each experiment/assay. Include laboratory and animal experiments, equipment used, and agents manipulated (e.g. biological organisms, human materials, and recombinant and synthetic nucleic acid molecules).

Histone deacetylase (HDAC)6 has been found to be overexpressed in lung cancer cell lines and is correlated with tumor cell progression. In order to evaluate the therapeutic potential of HDAC6-inhibition in lung adenocarcinoma, we will use lentiviral vector-mediated CRISPR/Cas9/sgRNA to knockdown HDAC6 gene in transgenic mouse models of lung cancer and in primary cultured mouse lung endothelial cells. We will package the 3rd generation lentiviral vector in the lab using HEK293 cells. Pipetting, centrifuging and flow cytometry will be done, and sharps will be used for mouse injections and tissue excision.

SCREENING QUESTIONS

Will **Viral Vectors** be used?	☒ YES ☐ NO	**(If YES, Complete VIRAL VECTORS)**
Will **Bacteria, Yeast, Fungi, Parasites, Viruses, toxins, or prions** be used?	☐ YES ☒ NO	**(If YES, Complete MICROORGANISMS, TOXINS, PRIONS)**

(Does not include viral vectors)

Will **Human or Non-Human Primate material** be used? ☐ YES ☒ NO **(If YES, Complete HUMAN AND NON-HUMAN PRIMATE MATERIALS…)**
(Does include tissues, blood, body fluids and cell lines)

Will **Animals** be used? ☒ YES ☐ NO **(If YES, Complete ANIMAL EXPERIMENTS and ANIMAL EXPERIMENTS PRECAUTIONS AND PROCEDURES)**

Will **Recombinant or Synthetic Nucleic Acids** be used? ☒ YES ☐ NO **(If YES, Complete RECOMBINANT DNA)**

NOTE: To expand/collapse all headings, right click on any heading and select "Expand/Collapse".

RECOMBINANT DNA

Use of Non-Viral Recombinant or Synthetic DNA

Note: To add rows, click on the table then click on the **"+"** on the right bottom corner. To delete rows, right click on the row and select "Delete Item".

Host for propagation or recipient of rDNA (e.g. E. coli K12, human cells, CHO cells)	Expression System or Transfer mechanism (e.g. plasmid expression vectors, baculovirus, etc.)	Name of expressed or silenced gene(s) (e.g. GFP, p53, etc.)	Nature of Insert (e.g. oncogene, reporter gene, etc.)	Animal use?
Primary murine cells, mouse models of lung cancer	pLentiCRISPR (lentivirally mediated CRISPR-cas9)	HDAC6 (silenced)	oncogene	☒ Yes ☐ No

VIRAL VECTORS

Note: To add rows, click on the table then click on the **"+"** on the right bottom corner. To delete rows, right click on the row and select "Delete Item".

Viral vector	Inserted expressed genes or silenced genes	Plasmids, packaging system, tropism	Viral vector source:	Animal use?
Lentiviral vector **If Other, specify:** *Click to enter text.*	**List specific genes:** Cas9, sgHDAC6 **List physiological function:** CRISPR-based silencing of oncogene ☐ Tracer, e.g. GFP ☐ Oncogene or proto-oncogene ☐ Toxin or allergen ☐ Tumor suppressor	**List specific name of plasmids/vectors:** pLentiCRISPR **Check all that apply:** ☒ Replication-incompetent ☒ Packaged in lab ☐ Purchased already packaged, ready-to-use ☐ VSV-G pseudotyped ☐ 2nd generation (lentiviral vector only) ☒ 3rd generation or higher (lentiviral vector only) ☐ Amphotropic ☐ Ecotropic	☒ Vendor name: Promega ☐ Collaborator name: *Click to enter text.*	☒ Yes ☐ No

MICROORGANISMS, TOXINS, PRIONS

HUMAN AND NON-HUMAN PRIMATE MATERIALS

- Please list human and non-human primate (NHP) materials used.

Please be specific e.g. human blood, human primary macrophages, established human cell line HEK293, human kidney tissue

Note: To add rows, click on the table then click on the **"+"** on the right bottom corner. To delete rows, right click on the row and select "Delete Item".

Human or non-human primate material	Material description	Source	Used in Animals
HEK293 cells (packaging)	☒ Known to be infected with an infectious agent. **Describe**: Contains Ad5 fragments ☐ Donors tested for HIV, HBV, HCV ☐ NHPs tested for herpes B ☐ Primary cells or tissues ☐ Induced pluripotent stem cells ☐ Human embryonic stem cells	☒ Vendor name: ATCC ☐ Collaborator name: *Click to enter text.* ☐ Biobank, specify the institution: *Click to enter text.*	☐ Yes ☒ No

RISK ASSESSMENT SECTION

Risk Assessment Discussion: Please discuss the potential biosafety risks associated with the agents or procedures *listed above.*

Potential biosafety risks associated with lentiviral vector include recombination of the vector into replication-competent virus or oncogenesis/other downstream effects after accidental self-inoculation. Potential biosafety risks associated with HEK293 cells include potential exposure to human bloodborne pathogens and tumorigenesis following accidental exposure. Although the target gene is a suspected oncogene, the gene is being silenced, not overexpressed. Exposure to CRISPR may have unintended downstream effects.

Potential routes of exposure include exposure to aerosols or to contaminated sharps (needles, scalpels, scissors).

LABORATORY WORK PRECAUTIONS AND PROCEDURES

Personal Protective Equipment (may be multiple selected)

Note: To add rows, click on the table then click on the "+" on the right bottom corner. To delete rows, right click on the row and select "Delete Item".

BSL1	BSL2	BSL2+	BSL3
☐ N/A ☐ Standard Lab PPE (lab coat, nitrile gloves, safety glasses)	☐ N/A ☒ Standard Lab PPE (lab coat, nitrile gloves, safety glasses) ☐ Other: *Click to enter text.*	☐ N/A ☐ Standard Lab PPE (lab coat, nitrile gloves, safety glasses) **Enhanced PPE**: ☐ Double gloves ☐ Sleeve covers ☐ Disposable lab gown ☐ Face/surgical mask ☐ Face shield ☐ Other: *Click to enter text.*	☐ N/A ☐ PAPR respirator ☐ N95 respirator with face shield ☐ N95 respirator with safety glasses ☐ Double gloves ☐ Sleeve covers ☐ Disposable lab gown ☐ Shoe covers ☐ Dedicated shoes ☐ Other: *Click to enter text.*

Aerosol/Droplet Generating Equipment Used with BSL2, BSL2+, or BSL3 materials

Note: To add rows, click on the table then click on the "+" on the right bottom corner. To delete rows, right click on the row and select "Delete Item".

Equipment	Controls
☒ Centrifuge ☐ N/A	☒ Aerosol-proof rotors or safety cups with lids
☐ Homogenizer ☐ N/A	☐ Used inside a biosafety cabinet
☒ Cell sorter ☐ N/A	☒ Used inside a biosafety cabinet
☐ Sonicator ☐ N/A	☐ Used inside a biosafety cabinet
☐ Lyophilizer ☐ N/A	☐ HEPA-filtered exhaust line
☐ Other: *Click to enter text.*	**Describe controls:** *Click to enter text.*

Sharps

- Which of the following sharps will be used in BSL2 labs with BSL2 materials?

  ☒ Needles ☒ Scalpels ☐ Pasteur pipettes ☐ Cryostat ☐ Microtome ☒ Other **scissors will be used to cut up mouse lung** ☐ None

- Explain how the sharps indicated above will be used: Self-retracting needles will be used for mouse injection, scalpels and scissors will be used for

dissection/tissue processing.

- Will sharps be used in BSL2+ laboratories with BSL2+ materials? ☐ YES ☒ NO
  If **YES**, explain:

- Will sharps be used in BSL3 laboratories with BSL3 materials? ☐ YES ☒ NO
  If **YES**, explain:

Shipping/Transporting

- Will you be transporting any microorganisms, cell lines, viral vectors or plasmid vectors listed in this protocol to another laboratory or to another institution (e.g. animal facility)? ☒ YES ☐ NO
  If **YES**, explain: Viral vector will be transported to animal facilty. Mouse tissue will be transported to the main laboratory.

  Will you be using a leak-proof primary container inside a rigid, closable secondary container for transport? ☒ YES ☐ NO

- Will you be shipping any human or non-human primate material (i.e. blood, tissues, cells), microorganisms, viral vectors capable of infecting human, animal or plant cells or causing human, animal or plant diseases listed in this protocol? ☐ YES ☒ NO
  If **YES**,
  Have you been trained to ship these materials (DOT or IATA)? ☐ YES ☒ NO

Disinfection and waste disposal

Note: To add rows, click on the table then click on the blue "+" on the right bottom corner. To delete rows, right click on the row and select "Delete Item".

Item	Spills	Biosafety cabinet, centrifuge, microscope stage	Liquid waste before drain disposal	Solid waste before off-site treatment	Sharps (needles, pasture pipettes etc.); syringes
Disinfection method: *Click to enter text.*	☒ 10% bleach ☐ Other: *Click to enter text.*	☒ 70% EtOH ☐ Other: *Click to enter text.* ☐ N/A	☒ 10% bleach ☐ Other: *Click to enter text.* ☐ N/A	☒ Biohazard bin ☐ Autoclaved ☐ Other: *Click to enter text.* ☐ N/A	☒ Sharps container ☐ N/A

Please list any additional lab-specific equipment or non-disposable items used with BSL2, BSL2+, or BSL3 materials and disinfection method: ☒ N/A

ANIMAL EXPERIMENTS

1. Will transgenic rodents be used? ☒ YES ☐ NO

   If **YES**, check all that apply: ☐ Created in-house ☒ Obtained from a vendor or collaborator

2. Will transgenic insects be used or generated? ☐ YES ☒ NO

   If **YES**, will insects host rDNA or rDNA modified microorganisms? ☐ YES ☐ NO

   If **YES**, describe: _____

List biological agents used in animals or check NOT APPLICABLE if biological agents will not be used in animals ☐ N/A

Note: To add rows, click on the table then click on the **"+"** on the right bottom corner. To delete rows, right click on the row and select "Delete Item".

List biological agent used in animals: e.g. human cell line, viral vector	**Dose & frequency**	**Exposure route**	**Animal**	**Animal Biosafety Level**
Agent: lentiviral vector (pLentiCRISPR) ☐ Wild type ☒ Recombinant	*Click to enter text.* ☒ One time ☐ Multiple	☐ IV ☒ IP ☐ IM ☐ SC ☐ oral ☐ IC ☐ IO ☐ IN ☐ Other: *Click to enter text.*	**Species:** mouse ☒ Transgenic ☐ Knockout ☐ Immunodeficient ☐ Other: *Click to enter text.*	☐ BL1N practices ☒ BL2N practices for 72 hrs. ☐ BL2N practices for life

ANIMAL EXPERIMENTS PRECAUTIONS AND PROCEDURES

Personal Protective Equipment and Disinfection

- ☒ All appropriate PPE as specified for the animal facility will be worn.
- Please check the disinfectants that you will use in the animal facility ☒ Clidox ☐ Other ☐ 70% EtOH ☐ 10% bleach

BL2N Procedures and Precautions

- Will different viral vectors or pathogens be co-injected or sequentially injected into the same animal? ☐ N/A

  ☐ YES ☒ NO If **YES**, please explain: _____

- How long will the animals be handled as if they were biohazardous, e.g. using BL2N procedures?

  ☒ 72 hours (if exposed to replication incompetent viral vectors)

  ☐ for the life of the animal (if exposed to primary human cells, established human cell lines, or human pathogens)

  ☐ for the life of the animal (if exposed to viral vectors carrying high risk genes or replication competent viral vectors)

- Will the animals be handled during the biohazardous period (72 hours or life of the animal) after exposure to the biological agent? ☐ YES ☒ NO
  If YES, please explain and indicate if a biosafety cabinet will be used: _____

- Check all BL2N practices that you will follow for the biohazardous period (either 72 hours post-exposure or for the life of the animal):

  ☐ Animals anesthetized prior to injection of biological agent

  ☒ Dosing with biological agent done in a biosafety cabinet

  ☒ Animal cages are changed by research staff during the biohazardous period

  ☒ Initial cage change performed using a biosafety cabinet for BL2N 72 hours

  ☐ All cage change performed using a biosafety cabinet for BL2N for the life of the animal

  ☐ Cages are labeled as BL2N

  ☒ Carcasses are double bagged and placed in the designated freezer for proper disposal

  ☒ Solid contaminated waste collected in an appropriate biohazard bin

  ☒ If needles will be used, I confirm needles will not be recapped

  ☒ If needles will be used, they will be disposed of after use in a red sharps container

PRINCIPAL INVESTIGATOR DUAL USE RESEARCH OF CONCERN (DURC) ASSURANCES

Does your research involve the use of any of the following agents and toxins that are subject to DURC policy? ☐ YES ☒ NO

- Botulinum neurotoxins (any quantity)
- Avian influenza virus (highly pathogenic)
- *Bacillus anthracis*
- *Burkholderia pseudomallei*

- *Burkholderia mallei*
- Foot-and-mouth disease virus
- *Francisella tularensis*
- Reconstructed 1918 influenza virus
- Rinderpest virus
- Toxin-producing strains of Clostridium botulinum
- Yersinia pestis
- Ebola virus
- Marburg virus
- Variola major and minor viruses

If **YES**, please indicate if your research with the agents listed above falls into any of the categories below:

1. Enhance the harmful consequences of a biological agent or toxin ☐ YES ☐ NO
2. Disrupt immunity or effectiveness of an immunization without clinical and/or agricultural justification ☐ YES ☐ NO
3. Confer to a biological agent or toxin, resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or toxin, or facilitate their ability to evade detection methodologies. ☐ YES ☐ NO
4. Increase the stability, transmissibility, or the ability to disseminate a biological agent or toxin. ☐ YES ☐ NO
5. Alter the host range or tropism of a biological agent or toxin ☐ YES ☐ NO
6. Enhance the susceptibility of a host population ☐ YES ☐ NO
7. Generate a novel pathogenic agent or toxin, or reconstitute an eradicated or extinct biological agent ☐ YES ☐ NO