NDSU NORTH DAKOTA STATE UNIVERSITY

NOTICE OF IBC APPROVAL

7/22/2019

Keerthi Nawarathna Electrical and Computer Engineering

B19032-Biomanufacturing of safe and non-viral CAR T-cells for cancer immunotherapy

Research location(s): Van Es 114, Ehly 202

The NDSU Institutional Biosafety Committee has reviewed and approved the above referenced protocol under the category:

EXEMPT rDNA NIH Guidelines Section III-F-1
Human blood and OPIM

Approval Date: 7/22/2019
Annual Update Due: 6/1/2020
Expiration Date: 7/21/2024

As project director, you are responsible for conducting the protocol as described, utilizing BSL-2 containment. The IBC retains the right to conduct inspections of the research facilities at any time.

Additional reporting to the NDSU IBC is required for this project:
• when making changes in personnel and/or any approved procedures, locations or agent(s). Submit the IBC Request for Change Form prior to implementation of the change(s). Please note that changes may require re-evaluation of the risk assessment and biosafety level for appropriate containment.
• in the event of significant problems concerning exposure or containment of the agent(s) involved. Submit the IBC Adverse Event Reporting Form within 24 hours of awareness of the event.
• if the study will continue beyond the approval period. Submit the Progress Report Form for IBC review approximately one month prior to the expiration date. In addition, annual status updates are required (submit the IBC Annual Update/Completion Report Form). You should receive a reminder approximately one month prior to each report date.

NOTE Report all exposure incidents (e.g., via skin puncture, mucous membrane, non-intact skin, or other means) immediately to the Safety Office at 701-231-7759 for evaluation and follow up. Also notify the IBC of the incident within 24 hours.

INSTITUTIONAL BIOSAFETY COMMITTEE
NDSU Dept 4000 | PO Box 6050 | Fargo ND 58108-6050 | 701.231.8908 | Fax 701.231.8098 | ndsu.research@ndsu.edu

Shipping address: Research 1, 1735 NDSU Research Park Drive, Fargo ND 58102

NDSU NORTH DAKOTA STATE UNIVERSITY

Institutional Biosafety Committee
Research Integrity and Compliance Office
Research 1-1735 Research Park Drive
www.ndsu.edu/ibc

Institutional Biosafety Application
Questions about this form? Call 231-8908
Submit form to ndsu.ibc@ndsu.edu

IBC USE ONLY

Protocol # B19032 Review ○ Designated ○ Full Board Approval Date 7/22/2019

Protocol Title: Biomanufacturing of safe and non-viral CAR T-cells for cancer immunotherapy

Principal Investigator Name: Keerthi Nawarathna

Department: Electrical and Computer Engineering

Email: dharmakeerthi.nawara@ndsu.edu

Phone: 701-231-7916

Funding Agency: National Institutes of Health

Anticipated Start and End Date: 09/01/2019- 09/30/2020

Other Approvals

IACUC Approval: No Protocol Number:

IRB Approval: No Protocol Number:

Radioisotopes- No Contact the Radiation Safety Committee

Biological Toxins, Nanomaterials, or Chemicals: No Contact the Laboratory and Chemical Safety Committee

USDA-APHIS or BRS Permit: No Please attach a copy of the applicable permit.

Research Team and Required Training

All research team members should be listed below. Training requirements are based on the agents/materials used in the protocol. Required training courses (www.citiprogram.org)

All protocols-**Basic Biosafety Training Course**
Protocols involving Recombinant or Synthetic Nucleic Acids-**NIH Recombinant DNA Guidelines Course**
Protocols involving Human blood, bodily fluids, tissues, or cells-**OSHA Bloodborne Pathogens Course**

IBC Office staff will enter training expiration dates

Add Name	Name	Role in Protocol	Basic Biosafety	NIH Recombinant DNA Guidelines	OSHA Bloodborne Pathogens
Remove Name	Vidura Jayasooriya	Graduate Researcher	3/7/2022	3/7/2022	3/7/2022
Remove Name	Beth Ringwelski	Graduate Researcher	4/1/2022	4/1/2022	7/15/2022
Remove Name	Glenn Dorsam	Co-investigator	2/6/2022	3/1/2021	1/23/2020
Remove Name	Keerthi Nawarathna	Principal Investigator	7/16/2022	7/16/2022	7/16/2022
Remove Name	Caleb Nathaniel Laney	Graduate Researcher	6/6/2020	5/31/2020	1/24/2020

Project Description

In terms that would be understandable to the general public, provide a brief overview of the goals and objectives of the project; citing references as applicable

Project Goals -*Briefly describe your research project goal(s).*

Chimeric Antigen Receptor T-cells (CAR T-cells), have shown a great potential as a therapeutic strategy for Acute Lymphoblastic Leukemia (ALL). PI has recently developed low-cost, mRNA based CAR T-cell manufacturing technology. The goal of this research is to apply the technique to manufacture CAR T-cells from commercially available blood samples (T-cells) and investigate the efficacy of manufactured CAR T-cells.In this study, we will not use actual ALL cells from patients; we will use commercially available K-562 cells (human lymphoblast, ATCC; CCL-243) which is used as an alternative for ALL. Please see ref 1 for more details.

Procedures-*Briefly describe your laboratory and field procedures involving biohazardous agents.*

Purchase commercially available blood samples (Innovative research, product id: IWB1K2E10ML) from healthy individuals who are 30 years or less.
Use commercially available reagent kit (Stemcell technologies, product info: https://www.stemcell.com/easysep-direct-human-t-cell-isolation-kit.html) and isolate T-cells from blood samples
Tansfect T-cells with mRNA molecules that produce CAR molecules and incubate (at 37C, 5% CO2)
Transfection by electroporation will be performed using a microfluidics device developed in the PI's lab
Electroporation is used small AC electric fields to produce small pores on T-cell membranes and transport external molecules (mRNA) into interior of cells
Incubate (at 37C, 5% CO2) transfected T-cells 2-30 days in the lab incubator
Mix engineered T-cells (CAR T-cells) with target cancer cells (ALL) (1:1) and incubate additional 2 days and study CAR T-cells' ability to kill ALL cells (cytotoxic assay).
Commercially available ALL cells (k562 cells from ATCC) will be purchased, cultured in the lab and used in the experiments.
Commercially available reagent kit will be used to perform the cytotoxic assay (Promega, product id: G1780).

References-*List any literature references relevant to this application.*

1. Birkholz, K., Hombach, A., Krug, C., Reuter, S., Kershaw, M., Kämpgen, E., Schuler, G., Abken, H., Schaft, N. and Dörrie, J., 2009. Transfer of mRNA encoding recombinant immunoreceptors reprograms CD4+ and CD8+ T cells for use in the adoptive immunotherapy of cancer. Gene therapy, 16(5), pp.596-604.
2. Barrett, D.M., Zhao, Y., Liu, X., Jiang, S., Carpenito, C., Kalos, M., Carroll, R.G., June, C.H. and Grupp, S.A., 2011. Treatment of advanced leukemia in mice with mRNA engineered T cells. Human gene therapy, 22(12), pp.1575-1586.

Study Type

Please check the appropriate box(es) that apply to the project described above and provide additional information as requested.

☒ Recombinant or Synthetic Nucleic Acids

Check the NIH Guidelines section that is applicable to this project.

☒ **Section III-F Exempt Experiments**

☒ III-F-1 *Experiments involving nucleic acids that cannot replicate in any living cell, cannot integrate into DNA, and do not produce a toxin with an LD50 of less than 100ng/kg body weight*

☐ III-F-2 *Experiments not conducted in organisms, cells, or viruses and that have not been modified or manipulated to render them capable of penetrating cell membranes*

☐ III-F-3: *Recombinant or synthetic nucleic acids that exist solely of the exact nucleic acid sequence from a single source that exists in nature*

☐ III-F-4, III-F-5 *Nucleic acids from a prokaryotic or eukaryotic host, when propagated only in that host (or closely related strain of the same species*

☐ III-F-6 *DNA segments from different species that exchange DNA by known physiological processes (must consult Appendix A of the Guidelines for lists of designated exchangers.*

☐ III-F-7 *Genomic DNA molecules that have acquired a transposable element, if the transposable element does not contain any recombinant or synthetic DNA*

☐ III-F-8 *Experiments that do not present a significant risk to health or the environment (You must consult Appendix C of the Guidelines for specific categories and exemptions. Examples include Escherichia coli K-12 host-vector systems, Saccharomyces host-vector systems, purchase or breeding of transgenic rodents.*

Hepatitis B Vaccine Series. *The Hepatitis B vaccine series is available and must be offered to all personnel who have routine exposure to human blood, or other potentially infectious materials. Please refer to the* **NDSU Exposure Control Plan** *or contact the* **Safety Office** *for more information.*

Please list the names of individuals that will have routine exposure to human blood, bodily fluids or tissues while working on this project.

Add Name	Name	Hepatitis B Vaccine Status?
Remove Name	Vidura Jayasooriya	Vaccinated
Remove Name	Beth Ringwelski	Vaccinated
Remove Name	Caleb Laney	Vaccinated

Project Site(s)

Please list all sites and locations to be used (e.g. locations used for culture, creation, analysis, manipulation and storage of biohazardous agents. Include Core Facilities and locations for animals and plants used in conjunction with this project).

Add Location	Building/Location	Room #	Activity/Use	Biosafety Level
Remove Location	Ehly	202	Research lab	BSL-2
Remove Location	Van Es	114	Research lab	BSL-2

Projects that have a containment level of BSL-2 or higher are required to have a laboratory specific Biosafety Manual. In addition all locations where BSL-2 or higher work is being conducted are required to have the space inspected by the NDSU Biological Safety Officer.

Does this project have a containment level of BSL-2 or higher? (•) Yes ( ) No

Biosafety Laboratory Inspection	Yes
Laboratory Specific Biosafety Manual	Pending
Laboratory Inventory of Infectious Agents or Toxins	Yes-on file in the lab

Containment

Describe containment measures to prevent occupational or public health exposure to and/or release of biohazardous agents to the environment

☒ **Aerosols-**Procedures that may generate aerosols will be used in this project.

Please identify all experimental procedures that may generate aerosols and describe how they will be minimized or contained. Examples of procedures include: grinding, sonicating, blending, shaking, vortexing, vigorous pipetting, centrifuging, intranasal inoculation of animals, and opening pressurized containers.

Aerosols may generate during centrifugation and pipetting in the isolation of T-cells. Care will be taken, especially ejecting the disposable pipette tip and avoid the the aerosol formation. Centrifuge tips will not be overfilled, and researchers will wipe the outside of the tubes with disinfectant after they are filled and sealed. Researcher will never use mouth pipetting during the assays.

☒ **Biological Safety Cabinet-**A BSC or another type of containment chamber will be used in this project.

Please indicate the location and type of containment cabinet/chamber being used.

Ehly hall 202

☐ **Section III-E IBC Notice Simultaneous with Initiation**

☐ **Section III-D IBC Approval Before Initiation**

☐ **Section III-C IBC, IRB and RAC Approval Before Initiation**

☐ **Section III-B NIH/OBA and IBC Approval Before Initiation**

☐ **Section III-A RAC Review and NIH Director and IBC Approval Before Initiation**

***Sub-Sections**-Please indicate applicable sub-section(s) of the NIH Guidelines*

Add Row	Source of DNA	Nature of inserted DNA Sequence(s)	Vector(s) Indicate antibiotic trait(s)	Host(s)
Delete Row	commercial vender	mRNA	No	Viruses

Please check the applicable boxes

☐ In vivo use

☐ Expression of a foreign gene

☐ Genes coding for the biosynthesis of molecules toxic for vertebrates

☐ Growth of large-scale (>10L) cultures containing Recombinant or Synthetic Nucleic Acids

Procedures-*Describe specific procedures involving the collection, creation, use, analysis and transport of recombinant and synthetic nucleic acids.*

We will purchase mRNA molecules from a commercial vendor. Vender will will ship molecules in frozen vials to the PI's lab. Researchers will add 1-10 ug/ml in the transfection solution and T-cells will be transfected with mRNA molecules.

Risks-*Describe the risks for human, animal or plant/environmental health posed by these materials and what safety measures will be taken.*

Risks to humans or plan health will be minimum because unused mRNA in the buffer will degrade quickly. All the graduate researchers work on th project will be properly trained to use mRNA, dispose mRNA after use.

☐ **Infectious Agents**

☐ **Dual Use Research of Concern (DURC) (e.g. botulinum toxin)**

☒ **Human Blood, Bodily Fluids, Tissues or Cell Cultures**

Add Line	**Description** (e.g. whole blood, spinal fluid, tissue, cell lines)	**Source** (e.g. hospital, university, federal repository , commercial vendor or colleague)	Product Number or Approximate Date of Acquisition
Remove Line	Whole blood	commercial vendor	IPLA-WB1 or similar

Collection and Transport. *Describe specific procedures involving the collection and transport of human blood, bodily fluids, tissues and human cell lines.*

PI will place an order on-line through the company's (Innovative research, Novi, MI) website and company will ship the samples overnight by FedEX.

Use and Analysis. *Describe specific procedures involving the use and analysis of human blood, bodily fluids, or cell lines.*

Use commercially available reagent kits and isolate T-cells.
Tansfect T-cells with mRNA molecules and incubate.
Mix engineered T-cells (CAR T-cells) with target cancer cells (ALL) and study CAR T-cells' ability to kill ALL cells.

Risks. *Describe risks for human, animal and environmental health posed by these materials, and what measures will be taken to ensure safety.*

All human blood products sold by Innovative Research are collected at FDA-licensed commercial donor centers within the US. Each unit is tested by an FDA-approved method for Human Immunodeficiency Virus RNA (HIV-1 RNA), Antibodies to Human Immunodeficiency Virus (Anti-HIV 1/2), Antibodies to Hepatitis C Virus (HCV), Hepatitis C Virus RNA (HCV RNA), non reactive for Hepatitis B Surface Antigen (HbsAg), and non-reactive by a screening test for syphilis.
Each member of the study team will be vaccinated (Hepatitis B).

Date of last certification 08/08/2019

☐ **Plants**-This project involves the use of plants.

Waste

Please check the types of waste that will be generated

☒ Sharps
☒ Liquids
☐ Solids
☐ Animal Carcass
☐ Plants
☐ Other

If other, please explain:

Disinfection/Decontamination-*Describe procedures for disinfection/decontamination of the various types of wastes that will be generated.*

We will add bleach to the cell samples (both T-cells and ALL samples) and store in the biological waste container.

Disposal-*Describe procedures for disposal of biohazardous wastes.*

We will contact NDSU for disposal.

Text

Personal Protective Equipment (PPE)-*Mark all PPE that will be used in project.*

Personal Protective Equipment	Lab Procedures	Human/Animal Procedures	Plant Procedures
Laboratory Coat	☒	☒	☐
Gloves	☒	☒	☐
Safety Glasses/Goggles	☒	☒	☐
Face Shield	☐	☐	☐
Surgical Mask	☐	☐	☐
Respirator*	☐	☐	☐
Disposable Lab Coat	☐	☐	☐
Booties	☐	☐	☐
Hair/Head Cover	☐	☐	☐
Other (specify)			

**All respirators, including N-95 respirators, require medical clearance and fit testing to confirm the appropriate fit and protection. Additional information on the Respirator Program can be found on the website or by contacting the Safety Office (231.7759)*

Principal Investigator Assurance

By submitting this application, I agree to:

☒ Conduct this project in compliance with all policies and directives of North Dakota State University, as well as all applicable US laws.

☒ Be adequately trained in good microbiological techniques

☒ Instruct and train laboratory and research staff in the practices and techniques required to ensure safety and the procedures for dealing with accidents and releases

☒ Inform the laboratory staff of the reasons and provisions for any precautionary medical practices advised or recommended (e.g. vaccinations or serum collection)

☒ Supervise laboratory and research staff to ensure that required safety practices and techniques are followed.

☒ Correct work errors or conditions that may result in the release of recombinant or synthetic nucleic acid materials or pathogens

☒ Ensure all laboratory and research staff have access to the protocols describing the potential biohazards and necessary precautions

☒ Maintain the integrity of physical containment (e.g. biological safety cabinets) and biological containment (e.g. host-vector system that preclude survival of the agent outside the lab).

☒ Maintain a current inventory of infectious agents and toxins on file.

The information within this application is accurate to the best of my knowledge.

Principal Investigator Signature Keerthi Nawarathna Digitally signed by Keerthi Nawarathna Date: 2019.03.28 11:22:24 -05'00'

The signature below certifies acknowledgement that the research outlined in this application is in keeping with standards set by your department/unit, all NDSU policies and that facility, equipment and personnel are appropriately committed to this project.

Chair, Head, Director or Dean Signature Benjamin D. Braaten Digitally signed by Benjamin D. Braaten Date: 2019.03.29 09:24:58 -05'00'

Institutional Biosafety Committee

The IBC has reviewed and approved this protocol.

IBC Chair GEORGE YOCUM Digitally signed by GEORGE YOCUM Date: 2019.07.22 08:48:35 -05'00' ***Date***

Submit by Email