Safety Module – Sample Protocol

Answer the protocol SmartForm pages as follows.

> **Tip:** Save time by cutting and pasting text from the protocol document to the protocol SmartForm pages.

Basic Information

Select appropriate safety review: Biosafety

Title of protocol: A Rapid Screening Assay Identifies Monotherapy with Interferon-ß and Combination Therapies with Nucleoside Analogs as Effective Inhibitors of Ebola Virus.

Short title: <Your Name> Effective Inhibitors of Ebola Virus

Summary of research:

To date there are no approved antiviral drugs for the treatment of Ebola virus disease (EVD). While a number of candidate drugs have shown limited efficacy in vitro and/or in non-human primate studies, differences in experimental methodologies make it difficult to compare their therapeutic effectiveness. Using an in vitro model of Ebola Zaire replication with transcription-competent virus like particles (trVLPs), requiring only level 2 biosafety containment, we compared the activities of the type I interferons (IFNs) IFN-α and IFN-ß, a panel of viral polymerase inhibitors (lamivudine (3TC), zidovudine (AZT) tenofovir (TFV), favipiravir (FPV), the active metabolite of brincidofovir, cidofovir (CDF)), and the estrogen receptor modulator, toremifene (TOR), in inhibiting viral replication in dose-response and time course studies. We also tested 28 two- and 56 three-drug combinations against Ebola replication. IFN-α and IFN-ß inhibited viral replication 24 hours post-infection (IC50 0.038μM and 0.016μM, respectively). 3TC, AZT and TFV inhibited Ebola replication when used alone (50-62%) or in combination (87%). They exhibited lower IC50 (0.98-6.2μM) compared with FPV (36.8μM), when administered 24 hours post-infection. Unexpectedly, CDF had a narrow therapeutic window (6.25-25μM). When dosed >50μM, CDF treatment enhanced viral infection. IFN-ß exhibited strong synergy with 3TC (97.3% inhibition) or in triple combination with 3TC and AZT (95.8% inhibition). This study demonstrates that IFNs and viral polymerase inhibitors may have utility in EVD. We identified several 2 and 3 drug combinations with strong anti-Ebola activity, confirmed in studies using fully infectious ZEBOV, providing a rationale for testing combination therapies in animal models of lethal Ebola challenge. These studies open up new possibilities for novel therapeutic options, in particular combination therapies, which could prevent and treat Ebola infection and potentially reduce drug resistance.

Principal Investigator: Principal Investigator <your number>
(This should be pre-populated; please do not change the PI to your name)

Protocol Team Members

Add yourself as a person involved in the design, conduct, or reporting of the research.

Add at least one other protocol team member.

Biosafety Submission

Select the following:

- Tissues, Blood, or Body Fluids
- Select Agents or Toxins
- Recombinant or Synthetic Nucleic Acids
- Animals

Tissues, Blood, or Body Fluids

Add the following information:

- Category: Humans
- Tissue, Blood or Body Fluid Type: Human Excretory Tissue
- Biocontainment level: BSL-2

Select Agents or Toxins

Add the following information:

- Select agent or toxin: Ebola Virus
- Biocontainment level: BSL-3

Biohazards

Update the storage and usage information for the following agents:

Human Excretory Tissue

- Storage Location: Chemical Storage Building
- Usage Location: Biomedical Lab

Ebola Virus

- Storage Location: Chemical Storage Building
- Usage Location: Biomedical Lab

Recombinant or Synthetic Nucleic Acids Usage

Select the following information:

- The deliberate transfer of drug resistance into organisms that do not acquire them naturally
- Viable genetically-modified (with recombinant or synthetic nucleic acids) microorganisms tested in whole animals

Recombinant or Synthetic Nucleic Acid Work Description

For each experiment, list genes, inserts, gene products, and key regulatory elements to be cloned:

We generated recombinant ZEBOV expressing enhanced green fluorescent protein (eGFP) from cDNA clones of full-length infectious ZEBOV, as previously described [32]. The eGFP reporter protein was expressed as an eighth gene, and the virus exhibited an *in vitro* phenotype similar to wild-type ZEBOV. Notably, *in vivo*, incorporation of GFP into wild-type ZEBOV results in some attenuation of disease [32]. All work with infectious ZEBOV was performed in biosafety level 4 (BSL4), at the National Microbiology Laboratory of the Public Health Agency of Canada in Winnipeg, Manitoba.

30,000 293 T cells were seeded in 96-well plates in 100µL DMEM with 10% FBS. 24 hours thereafter, the medium was replaced with 100µL DMEM with 10% FBS containing ZEBOV-GFP at an MOI of 0.1. 24 hours post-infection, the medium was removed and replaced with 200µL of DMEM with 5% FBS, or 190µL DMEM with 5% FBS and 10µL of single or combinations of drugs. eGFP fluorescence was measured 3 days post-infection using a Synergy HTX Multi-Mode Microplate Reader (BioTek).

Animals

Identify the species to be used: Mouse

Risk Group and Containment Practices

What is the highest risk group level of the biological agents and materials you will use in the proposed research? RG-2

What are the highest biosafety containment practices required for the research activities covered by this protocol?

Under NIH Guidelines rDNA or synthetic nucleic acids:

- Physical Containment: BL2
- Research Involving Animals: BL2-N

Exposure Assessment and Protective Equipment Page

Describe consequences of exposure or release of agents used to humans, animals, and plants.

All personnel accidental exposures will be immediately reported to supervisor.

Indicate the personal protective equipment that will be used:

- Lab Coats
- Eye Protection
- Gloves
- Other: Face Masks

Dual Use Research of Concern Page

Dual use experiment categories used in this research:
Disrupts immunity or the effectiveness of an immunization against the agent or toxin without clinical or agricultural justification.

Explain why you believe this protocol is or is not dual use research of concern:
This protocol is dual use research of concern because it has the potential to disrupt immunity.

Waste Management

Describe the process for decontaminating biological waste:
Spills will be handled as per instructions in the Biosafety Office SOP Biohazard Spill Cleanup. Briefly, spill will be covered with paper towels and the towels are soaked with a 70% ethanol. Spill is allowed to soak for approximately 20 minutes before discarding materials in biohazard bag/box. Surfaces will be decontaminated with 70% ethanol. All disposable materials used for the cleanup (paper towels, gloves, etc.) will be discarded in a biohazard bag.

Describe the plans in the event of a biological accident:
Contaminated solid waste: Disposable solid waste will be placed in the biohazard autoclavable bags/boxes

Carcasses of infected/exposed animals: infected carcasses/tissues will be double bagged and placed in the vivarium carcass cold room, where vivarium personnel will decontaminate and dispose as per their standard SOPs.

Contaminated sharps: Contaminated sharps will be placed in a "sharp containers" which will be securely closed before placing it in the autoclavable boxes.