Column E Explanation

1. Registration Number: Armed Forces Radiobiology Research Institute, Certificate #51-F-0003

2. Number of animals used in this study: 132

3. Species (common name) of animals used in the study: Pigs

4. Explain the procedure producing pain and/or distress.

   The aims of this study are:

   1. To study a Gottingen minipig model of hematopoietic acute radiation sickness (H-ARS) for drug screening relevant to mass radiation casualty-scale treatment.
   2. To develop a pediatric animal model of acute radiation sickness (ARS).
   3. To study the effect of supportive care on survivability of irradiated Gottingen minipigs.

   Pain is not expected from the irradiation procedure itself but the sequel of radiation exposure at the levels used in this study can cause pain and distress. Animals are sedated with telazol during the irradiation procedure to reduce stress. 7-10 days post-irradiation various changes can occur in the body (e.g. immune system suppression, bone marrow suppression, gastrointestinal upset, etc.). These changes can potentially lead to bacterial infections, fever, anorexia, bleeding, nausea, vomiting, constipation and diarrhea which can cause pain and distress in the animal.

   Antibiotics, antifungals, food supplements, probiotics, fiber supplements and oral electrolyte supplements were used in this study for minimal symptomatic supportive care. Around 21-28 days post-irradiation, the animal's immune system, bone marrow and gastrointestinal system typically starts recovering and they start feeling better. If the animals do not recover or reach a point where pain and distress cannot be medically managed, they were humanely euthanized. Animals were closely monitored post irradiation multiple times a day, round the clock, until they completely recovered from ARS.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

   U.S. Food and Drug Administration (FDA) under the "Two Animal Rule" require that new radiation countermeasure drug must be tested in 2 animal species before testing it on human subjects and is the main bottleneck for drug licensure and commercialization. In spite of the many promising drug candidates developed for the ARS and proven successful in rodents, only one drug (G-CSF, Neupogen®) has been approved by the FDA for use in nuclear/radiological emergency under an Emergency Use Authorization (EUA). Gottingen minipigs can be used as non-rodent model of hematopoietic ARS (H-ARS) for the evaluation, development and licensure of radiation mitigators and therapeutics for use in a mass casualty scenario.

   Military personnel can be potentially exposed to radiation during their duty (assembling, disassembling, stockpiling of nuclear weapons, cleanup of facilities, disposal of radioactive materials, civilian defense, etc). So far, only four drugs specifically designated for use in radiation emergency are present in the Strategic National Stockpile. Limited availability of a sufficient number of well-characterized animal models to test radiation countermeasures had been indicated as one of the main bottlenecks for advanced drug development. Developing large animal models for testing of countermeasures to radiation exposure and identification of the patho-physiological mechanisms underlying the development of ARS are fundamental to clinical management.

   Very little information is available on the ARS in children, except for the well-known increased risk for cancer development. Because children are still in an active growing phase, the effects of radiation are expected to be more severe than for adults. Furthermore, radiation countermeasures developed for adults cannot be assumed to be effective or safe for children without prior testing, due to the fact that organs of children are immature, and their physiological and metabolic features still developing. Consequences for administration of adult drugs to children include potential differences in drugs' pharmacokinetics/pharmacodynamics and mechanism of action, over- or under-medication and unanticipated adverse events.

   Minimal supportive care provided to animals in this study mimics a mass radiation causality situation

in which the exposed person will not have access to the advanced medical care. They may not have access to advanced medical care requiring hospitalization like prescription controlled analgesics (opioids), intravenous antibiotics, fluid infusion or blood transfusion. The use of advanced medical care like blood/fluid transfusion would contradict with the aim of this study and can interfere with the test results.

A thorough search of databases: (1) Johns Hopkins Center for Alternatives to Animal Testing (Altweb): http://altweb.jhsph.edu/; (2) **AGRICOLA:** http://agricola.nal.usda.gov/ , (3) **PubMed:** http://www.ncbi.nlm.nih.gov/pubmed/ revealed no animal alternatives for radiation countermeasure study.

Column E Explanation

1. Registration Number: Armed Forces Radiobiology Research Institute, Certificate #51-F-0003

2. Number of animals used in this study: 20

3. Species (common name) of animals used in the study: Non-human Primates

4. Explain the procedure producing pain and/or distress.

Nuclear proliferation, terrorist activity, and the distribution of nuclear and radioactive materials through underground networks make incidents involving radiation injuries very likely. The principal purpose of this study is to develop countermeasures for acute radiation sickness (ARS) that could be used in case of nuclear disaster. The part of this study is also focused on determining the effects of radiation countermeasures on the immune and hematopoietic systems.

Pain is not expected from the irradiation procedure itself but the sequelae of radiation exposure at the levels used in this study can cause pain and distress. Animals are sedated with ketamine during the irradiation procedure to reduce stress. 7-10 days post-irradiation various changes can occur in the body (e.g. immune system suppression, bone marrow suppression, gastrointestinal upset, etc.). These changes can potentially lead to bacterial infections, fever, anorexia, bleeding, nausea, vomiting and diarrhea etc which can cause pain and distress in the animal. There were 2 arms of this study:

- Minimal supportive care – This arm mimics a mass radiation casualty situation in which the exposed person will only have access to over the counter medications. Animals in this group received symptomatic treatment including tylenol, oral fluid supplementation, loperamide, fresh fruits, and vegetables.
- Full supportive care – This arms mimics a situation of radiation casualty in which the exposed person will have full access to medical care in a hospital setting. Animals in this group received symptomatic treatment including tylenol, rimadyl, buprenorphine, topical bupivacaine, fluid infusion, blood transfusion, antibiotics, ondansteron, sucralfate, lomotil, loperamide, fresh fruits and vegetables.

Animals in both these groups either received the test drug or control drug for radiation countermeasure. Around 21-28 days post-irradiation, the animal's immune system, bone marrow and gastrointestinal system typically starts recovering and they start feeling better. If the animals do not recover or reach a point where pain and distress cannot be medically managed, they were humanely euthanized. Animals were closely monitored post irradiation multiple times a day round the clock until they completely recovered from ARS. Video monitoring was also used to closely monitor the animals.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Nonhuman primates are necessary for the pre-clinical development of a radiation countermeasure drug candidate intended for use in humans because drug metabolism and physiology are very similar between humans and nonhuman primates. Testing a drug with potential for human application in nonhuman primates ensures safety and specificity prior to the drug entering into the clinic for human trials. Rhesus macaques are the model of choice for investigations of radiation injury and countermeasures because of the large database available from the existing literature that allows for robust comparison. US Food and Drug Administration (FDA) has accepted rhesus macaques as the appropriate animal model for pilot and pivotal efficacy testing of radiation countermeasures under the Animal Efficacy Rule, where efficacy testing cannot be performed in humans.

A thorough search of databases: (1) Johns Hopkins Center for Alternatives to Animal Testing (Altweb): http://altweb.jhsph.edu/; (2) **AGRICOLA:** http://agricola.nal.usda.gov/ , (3) **PubMed:** http://www.ncbi.nlm.nih.gov/pubmed/ revealed no animal alternatives for radiation countermeasure study.

The minimal supportive care arm of the study mimics a mass radiation casualty situation in which the exposed person will not have access to advanced medical care and prescription analgesic medications. They may only have access to over the counter medications (like Tylenol). The use of prescription medications like analgesics, antibiotics, anti-diarrheal, anti-emetics and blood/fluid transfusion would

contradict with the aim of this arm of the study and can interfere with the test results.

Animals in the full supportive care arm of the study usually do not feel pain and distress as they can receive full supportive care post irradiation based on the clinical signs. Full supportive care includes analgesics, antibiotics, antipyretics, anti-emetics, antiseptics, antidiarrheal, fluid infusion and blood transfusion. If the animals did not recover or reach a point where pain and distress cannot be medically managed, they were humanely euthanized.

Column E Explanation

1. Registration Number: Armed Forces Radiobiology Research Institute, Certificate #51-F-0003

2. Number of animals used in this study: 36

3. Species (common name) of animals used in the study: Non-human Primates

4. Explain the procedure producing pain and/or distress.

The principal purpose of this study is to evaluate dosimetric technology to determine the radiation dose and acute-radiation sickness (ARS) outcome. Supporting information such as selection of hematological and proteomic analysis markers in blood will help identify the usefulness or problems associated with the technology being tested for detection of early response after mass-casualty irradiation scenarios.

Pain is not expected from the irradiation procedure per se but the sequelae of radiation exposure at the levels used in this study can cause pain and distress. Animals are sedated with Ketamine during the irradiation procedure to reduce the stress. 7-10 days post-irradiation various changes can occur in the body (i.e., immune system suppression, bone marrow suppression, gastrointestinal upset, etc.). These changes can potentially lead to bacterial infections, fever, anorexia, bleeding, nausea, vomiting and diarrhea etc. which can cause pain and distress in the animal. There were 2 arms of this study:

- Minimal supportive care – This arm mimics a mass radiation casualty situation in which the exposed person will only have minimal access to medical care. Animals in this group received symptomatic treatment including buprenorphine, bupivacaine, famotidine, cerenia, ondansteron, TUMS™, loperamide, diphenoxylate, oral fluid supplementation, carprofen, fresh fruits, and vegetables.
- Full supportive care – This arm mimics a situation of radiation casualty in which the exposed person will have full access to medical care in a hospital setting. Animals in this group received symptomatic treatment including antibiotics, fluid infusion and blood transfusion in addition to the items listed in the minimal supportive care group.

Around 21-28 days post-irradiation, the animal's immune system, bone marrow and gastrointestinal system starts recovering and they typically start feeling better. If the animals did not recover or reach a point where pain and distress cannot be medically managed, they were humanely euthanized. Animals were closely monitored post irradiation multiple times a day round the clock until they completely recovered from ARS. Video monitoring was also used to monitor the animals.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Development and validation of early-response radiation injury biomarkers is critical for effective triage and medical management of irradiated individuals. Radiological terrorist attack or accidental mass-casualty exposures are highly possible. In such events, medical countermeasure resources may be very limited, which means that having a minimal supportive care might be the only available option in triage.

Military Relevance. Military personnel responding to such emergencies can be exposed to radiation. Radiation management of such events requires rapid and accurate biodosimetry with high precision to determine risk for morbidity and mortality.

In addition, assessment of a population's exposure to other radiation threats, such as nuclear accidents and terrorism mass-casualty scenarios addresses the need for a "Clinical Radiological Biodosimetry" system to provide physicians with the ability to triage radiation victims, make appropriate treatment decisions, and reduce uncertainties associated with the variability of individual response to radiation exposure.

These studies are scientifically justified based on the national interest to identify, optimize, and validate FDA-approved biodosimetry devices for potential radiological threats including mass-casualty incident.

The project is focused on studying biodosimetric endpoints using a nonhuman primate radiation dose-response model, and to investigate the correlation between these endpoints and dose, ARS response severity response, and survival. Rhesus macaque model represent the "gold standard" for most acute radiation and medical countermeasure studies because of the close evolutionary relation to humans and similar effects of supportive care. Research findings have *potential applications to humans as demonstrated in recent DTRA Technical Report (*DTRA-TR-14-031, "Mathematical Models of Human Hematopoiesis Following Acute Radiation Exposure") *and paper published by Malt'sev and colleagues based on data collected in Chernobyl radiation accident victims (*Mal'tsev, VN, Ivanov, AA, Mikhailov, VF, and Mazurik, VK. The individual prognosis of the gravity of the outcome of radiation disease on immunological indexes. 2006. Radiat. Biol. Radioecol. 46:2, 152-158).

There are no alternative procedures for irradiation because it is a unique stimulus that cannot be otherwise duplicated. The endpoint currently mandated by the FDA for approval of radiation countermeasures is mortality. Moribundity was used as a surrogate for mortality, and euthanasia was used in order to minimize pain and distress, using an extensive set of criteria.

A thorough search of databases: (1) Johns Hopkins Center for Alternatives to Animal Testing (Altweb): http://altweb.jhsph.edu/; (2) **AGRICOLA:** http://agricola.nal.usda.gov/ , (3) **PubMed:** http://www.ncbi.nlm.nih.gov/pubmed/ revealed no animal alternatives for dosimetry investigation involving total body irradiation.

Minimal supportive care arm of the study mimics a mass radiation causality situation in which the exposed person will not have access to the advanced medical care. They may not have access to advanced medical care requiring hospitalization like intravenous antibiotics, fluid infusion or blood transfusion. The use of advanced medical care like antibiotics and blood/fluid transfusion would contradict with the aim of this arm of the study and can interfere with the test results.

Animals in the full supportive care arm of the study usually do not feel pain and distress as they can receive full supportive care post irradiation based on the clinical signs. Full supportive care includes analgesics, antibiotics, antipyretics, anti-emetics, antiseptics, antidiarrheal, fluid infusion and blood transfusion. If the animals do not recover or reach a point where pain and distress cannot be medically managed, they were humanely euthanized.