Column E Explanation

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

2. Number of animals used in this study: 48

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

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

The Gottingen minipig model is being characterized for advanced drug development. The “two animal rule” requires that the critical characteristics of the animal model used for efficacy testing must be identified, and the pathophysiology of mechanisms of radiation injury must be reasonably well-understood. To gather sufficient information animals will be exposed to lethal and sublethal doses of whole-body gamma radiation (0.6 Gy/min) utilizing the cobalt facility. The gray (symbol: Gy) is the SI unit of absorbed radiation dose of ionizing radiation (for example, X-rays), and is defined as the absorption of one joule of ionizing radiation by one kilogram of matter (usually human tissue).

Irradiation itself is not a painful process but it induces various changes in the body (i.e., vomiting and nausea, changes in hematology cells numbers, etc.). Although radiation does not induce pain, animals in these experiments might experience pain and distress prior to death because of sequelae. Radiation compromises the immune system. As a result of a compromised immune response, various types of infections can initiate and become painful. The sequelae of nausea, vomiting, and diarrhea may cause pain and distress as observed in humans in the early post-irradiation period, when lethal doses are used.

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.

To study the efficacy of any radiation countermeasure in modulating survival, and to investigate its mechanism of action, one needs to irradiate animals. Irradiation itself is not a painful process; in fact, it can be analgesic (Teskey, G. C., and M. Kavaliers. 1984. Ionizing radiation induces opioid-mediated analgesia in male mice. Life Sci 35:1547-1552), but it induces various changes in the body, and kills hematopoietic and other radiosensitive cells. In irradiated animals, the immune response is compromised, and opportunistic infections may ensue.

Irradiated animals die due to compromised immune responses and opportunistic infections. The percentage of surviving animals is the indicator of the efficacy of a countermeasure. We cannot give systemic anesthetic and/or analgesic agents to animals after the irradiation procedures, since they are known to interact with the immune system (Jacobsen, K. 0., V. Villa, V. L. Miner, and M. H. Whitnall. 2004. Effects of anesthesia and vehicle injection on circulating blood elements in C3H/HeN male mice. Contemp Top Lab Anim Sci 43:8-12), and would confound the correlation of radiation dose with incidence of moribundity, resulting in a waste of animals.

The endpoint currently mandated by the FDA for approval of radiation countermeasures under the Animal Efficacy Rule is moribundity or mortality. Moribundity will be used as a surrogate for mortality, and euthanasia will be used in order to minimize pain and distress.

Column E Explanation

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

2. Number of animals used in this study: 20 (Study A) and 14 (Study B)

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

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

   To evaluate the pharmacokinetics of GT3 as well as the efficacy of GT3 in enhancing survival of irradiated NHPs (Study A) and to evaluate the efficacy of ALXN4100TPO in enhancing survival of irradiated NHPs (Study B) the animals must be exposed to whole-body gamma radiation (0.6 Gy/min) utilizing the cobalt facility. The gray (symbol: Gy) is the SI unit of absorbed radiation dose of ionizing radiation (for example, X-rays), and is defined as the absorption of one joule of ionizing radiation by one kilogram of matter (usually human tissue).

   There are no alternative procedures for irradiation because it is a unique stimulus/stress that cannot be otherwise duplicated. Radiation itself does not cause pain or distress. Nevertheless, the sequelae of nausea, vomiting, and diarrhea causes pain and distress, as seen in humans in the early post-irradiation period, when high doses are used. Although radiation does not induce pain, animals in these experiments might experience pain and distress prior to death because of hematological and gastro-intestinal damage.

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.

   Study A and B: It is expected that GT3 (Study A) or ALXN4100TPO (Study B) will provide some relief from pain and or discomfort due to the sequelae of irradiation by its protective effect and by the possibility that it will advance hematopoietic recovery in some or all of the GT3-treated and irradiated primates (Study A) or ALXN4100TPO-treated and irradiated primates (Study B). Along these lines, opioid analgesics are immunomodulatory (Pruett *et al,* 1992, Pasotti *et al,* 1993, Carr *et al,* 1994) and will not be used to relieve pain or distress. Non-narcotic analgesics such as indomethacin are anti-inflammatory and could interfere with the inflammatory responses of the antibacterial activity of hematopoietic tissues. Analgesics cause adverse effects on undamaged hematopoietic cells, (Hollaender, 1960) and interfere with nicotinamide-adenine dinucleotide phosphate (NADPH+ oxidase), a key polymorphonuclear leukocyte enzyme that is involved in the ability of the cells to undergo phagocytosis of bacteria (Moon *et al*, 1986). Because of these observations, we do not intend to use analgesics as they will interfere with the purpose of the research effort.

   References:

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   Pasotti, D., A. Mazzone, S. Lecchini, G.M. Frigo, and G. Ricevuti (1993). Influenza dei peptidi oppioidi sui granulociti del sangue periferico. [The effect of opioid peptides on peripheral blood granulocytes.] "Riv. Eur. Sci. Med. Farmacol" **15**: 71-81.

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