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Past and Future Work on Radiobiology Mega Studies: A Case Study at Argonne National Laboratory

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Abstract

Between 1952 and 1992 more than 200 large radiobiology studies were conducted in research institutes throughout Europe, North America and Japan to determine the effects of external irradiation and internal emitters on the life span and tissue toxicity development in animals. At Argonne National Laboratory, 22 external beam studies were conducted on nearly 700 beagle dogs and 50,000 mice between 1969 and 1992. These studies helped to characterize the effects of neutron and gamma irradiation on lifespan, tumorigenesis, and mutagenesis across a range of doses and dosing patterns. The records and tissues collected at Argonne during that time period have been carefully preserved and redissemated. Using these archived data ongoing statistical work has been done and continues to characterize quality of radiation, dose, dose rate, tissue, and gender specific differences in the radiation responses of exposed animals. The ongoing application of newly developed molecular biology techniques to the archived tissues has revealed gene specific mutation rates following exposure to ionizing irradiation. The original and ongoing work with this tissue archive is presented here as a case study of a more general trend in the radiobiology mega studies. These experiments helped form the modern understanding of radiation responses in animals, and continue to inform development of new radiation models. Recent archival efforts have facilitated open access to the data and materials produced by these studies and so a unique opportunity exists to expand this continued research.

Introduction

The development of atomic energy, threat of nuclear war, concerns about environmental radioactive contamination, potential dangers of space travel, and other concerns motivated a world wide effort to understand the effects of exposure to radiation on living tissues during the second half of the 20th century. Billions of dollars in funding were granted to life-long studies on large animal populations exposed to a multitude of internal and external radiation treatments in national laboratories throughout Europe, North America, and Japan. The health of these experimental animals was closely tracked and recorded. Many tissue and tumor samples were preserved for future study.

These radiobiology mega studies helped to build the modern model of radiation effects in animals. These studies focused primarily on the effects of exposure to external beam gamma and neutron irradiation. However, treatment outcomes of other types of ionizing and non-

ionizing radiation (such as X-rays and UV) were studied in animals as well along with radiation injury caused by inhaled and injected radionuclides such as radon, plutonium, thorium or others. While many endpoints and treatment variations were tested, the majority of these studies focused on the effects of dose, dose rates, and fractionation on life shortening and tumorigenesis. From these model studies scientists have ascertained the relative biological effectiveness (RBE) of various radiation treatments on individual tissues and overall lifespan.

Since their completion, archival efforts have sought to preserve and make available the data and samples from these studies. Currently there are multiple projects working to re-analyze and publish the documentation, data, and records of tissues generated by these studies online. This effort should facilitate ongoing statistical research, aid the development of more sophisticated models of radiation toxicity, and enable the use of modern molecular methods, immunohistochemistry, and genetic techniques to elucidate the effects of radiation mechanistically at the molecular level.

A few reviews of these radiobiology mega studies have been published since their completion. The most comprehensive of these is the Description of Participating Institutions and Studies compiled by the International Radiobiology Archive (IRA) (Gerber et al., 1996). Their report details the purpose, experimental details, and published references for 117 studies at 18 laboratories in Europe, 114 studies at 11 laboratories in the United States, and 33 studies at 12 laboratories in Japan. Other sources review specific radiobiology study categories in depth. For example, Roy Thompson's Book, Life Span Effects of Ionizing Radiation in Beagle Dogs, elucidates the most relevant findings and references from the Beagle dog experiments conducted in various national laboratories across the United States (Thompson, 1989). Many more sources detail the results of individual experiments.

While the previously referenced reviews are excellent sources on radiobiology mega studies, their static nature cannot capture the ongoing efforts to preserve and study the radiobiology archives. Separate programs by the European Radiobiology Archive (ERA) (Tapio et al., 2008), Japanese Radiobiology Archive (JRA), and the National Radiobiology Archives (NRA) (Watson, 2010) have worked hard to preserve and categorize the studies under the umbrella of the IRA (Gerber et al., 1999). While the IRA no longer functions as a distinct institution, continuing efforts by the ERA (Tapio et al., 2008) and the Woloschak Laboratory (Woloschak, 2010) have carried on its legacy by digitizing mega study records and making them available online.

The goal of this document is to characterize the ongoing archival and experimental efforts in their historical context. While a complete review of the original and ongoing work of all of the radiobiology mega experiments is beyond the scope of a single paper, this paper seeks to highlight the thread of research conducted using external irradiation at Argonne National laboratory between 1969 and 1992. The external beam studies have been chosen as a subject of this review because they represent the largest set of studies conducted at Argonne National Laboratory and represent the bulk of the archived tissues in the collection at Northwestern University.

The Argonne external beam studies performed in the 1970's and 80's comprised three blocks of experiments which consisted of 3 studies conducted on 4,900 beagle dogs, 11 'Janus' studies exploring the effects of neutron irradiation on 50,000 mice, and 2 mutagenesis studies conducted in mice. Together, these account for 16 of the 43 external beam studies conducted at National Laboratories in the United States and Canada and 25% of all research animals that participated in radiobiology experiments. 78 more external beam studies were conducted in major laboratories throughout Europe and Japan. Together these

studies represent a significant bulk of the radiobiology research conducted in the 20th century (Gerber et al., 1996). The Argonne external beam studies, both by history and ongoing research, resemble the efforts of other major archives.

While this review will not attempt to be exhaustive, it is the authors' hope to cultivate an excitement for the unique potential of archived resources in radiobiology to continue to shape science's understanding of the effects of irradiation on living organisms and to inspire a new generation of researchers to exploit that potential using novel statistical techniques and the full potential of modern molecular biology.

Argonne Beagle Dog Studies

The Argonne Beagle Dog Experiments were comprised of fifteen distinct experiments conducted between 1961 and 1991 on 4,900 Beagle dogs at Argonne National Laboratory in Illinois. Beagle dogs were chosen at that time for their ready availability, easy management, optimum size and lifespan, and physiological similarities to humans (Thompson, 1989). The studies focused on health and safety risks of ^{90}Sr , ^{144}Ce , and ^{137}Cs injection (Nikula et al., 1996), and whole body ^{60}Co gamma ray exposure (Carnes and Fritz, 1991, 1993). In this review we will focus on only those three studies which examined external gamma ray exposure (Figure 1).

The materials and results of the Argonne Beagle Dog Experiments dog tissue archive have been newly restored by the Woloschak laboratory under a grant from the U.S. Department Of Energy (DOE) (janus.northwestern.edu/dog_tissues). This work has made it possible to search for dog data and samples by treatment conditions, demography, and pathological observations. The Woloschak laboratory has also committed some of their resources to make the paraffin embedded tissue samples and original source data available upon request. Finally, the summary documentation and links to original ANL reports have been made available through the group wiki (janus.northwestern.edu/radbio).

Major findings of the beagle dog studies

The dogs involved in the following studies were all exposed to whole body external beam radiation while housed in one-dog kennels like those pictured in Fig. 1. After all exposures were complete, the dogs were transferred to outdoor kennels where they lived with 2 other dogs. The animals were given regular medical care which was focused on maintaining the dog's health, but not treating pathologies as they developed (Thompson, 1989).

Continuous exposure to gamma irradiation on dogs 400 to 500 days old had two distinct effects in dogs depending on the severity of the dose. All 72 dogs exposed to high doses, over 26 cGy day^{-1} , suffered acute deaths from septicemia less than 100 days after their first exposure. Acute deaths were not observed in 200 dogs exposed to doses under 1.9 cGy day^{-1} . Instead, they suffered from late effect toxicities, high tumor counts, and lifespan reduction. The effect of intermediate doses on the 53 dogs exposed to doses between 1.9 and 26 cGy day^{-1} resulted in a mix of early and late effects (B. Carnes and T. Fritz, 1993).

A follow up study which limited the total dose received across a range of dose rates found a similar threshold of early blood effects vs. late effects related to life shortening and tumorigenesis. It is noteworthy that at cumulative doses under 4.5 Gy no acute deaths were observed regardless of the dose rate ($3.8 - 26.3 \text{ cGy day}^{-1}$). On the other hand, even at the lowest cumulative dose, 4.5 Gy , neoplastic deaths became more prominent and the average lifespan of dogs was shortened compared to non-irradiated controls (B. Carnes and T. Fritz, 1991). Taken together, these gamma irradiated beagle lifespan studies lead to an understanding about the effects of whole body exposure to external beam radiation

segregating lethal early blood effects that occurred over a certain threshold dose and dose rate from the increased tumorigenesis and decreased lifespan that occurred at doses and dose rates falling below this threshold.

Argonne Mouse Mutagenesis Studies

Between 1971 and 1986 a series of studies were conducted at Argonne National Laboratory to determine the mutagenic and reproductive effects of low doses of neutron and gamma irradiation on young adult male B6CF1 mice. The mice were housed 5 to a cage and placed into one-pint polyethylene containers for whole-body external beam radiation exposure. Neutron irradiation was performed using the 0.85 MeV Janus reactor and gamma irradiation was performed using a cobalt 60 multisource irradiator.

In the first study Argonne researchers examined the effects of acute neutron and gamma irradiation on dominant lethal mutation rates, chromosomal translocation, testis size and spermatogenesis. They found that total doses of fission spectrum neutron irradiation ranging from 1 to 40 cGy and gamma ray irradiation ranging from 22.5 to 145 cGy caused predictable increases in chromosome translocation, abnormal epididymal sperm, and testicular weight loss. The RBE of neutron irradiation was higher at lower doses. At doses over 10 cGy the RBE of neutron irradiation was between 5 and 6 for dominant lethal mutations, abnormal sperm production, and testicular weight losses. At doses less than 10 cGy the RBE rose to 12 for dominant lethal mutations, 8 for abnormal sperm production, and 8 for testicular weight loss (Grahn et al., 1984).

A follow up study compared the effects of regular weekly exposures to single exposures on pre and post implantation fetal deaths for both neutron and ^{60}Co gamma ray irradiation. Fractionated neutron irradiation at total doses from 0.125 to 0.67 cGy administered weekly had a higher RBE for inducing post-implant fetal losses than the same total dose administered as a single fraction. The RBE rose from 5 to 12 for single vs. fractionated irradiation. The rising RBE of neutron versus gamma irradiation at low doses was a consistent trend in these studies. Where the effects of gamma irradiation were tempered by fractionation, neutron irradiation was unaffected or even more effective following fractionation, a trend that was also observed in the Janus Mouse studies detailed below (Grahn et al., 1986).

Argonne Janus Mouse (JM) Studies

The Argonne JM studies centered on irradiations performed with a particular source, the Janus reactor, capable of delivering both gamma and neutron irradiation. Operating from 1969 until 1992, the Janus reactor was employed for 11 major studies on 49,000 animals that included acute, fractionated, and protracted exposures, variable dose rate exposures, low dose studies, and radioprotector testing on B6CF1 mice and a control experiment on White-Footed field deer mice (*Peromyscus leucopus*). Cumulative gamma ray doses ranged from 90 to 5,111 cGy throughout the experiments, while neutron doses ranged from 1–470 cGy. As in the mutagenesis studies, the mice were housed 5 to a cage and exposed to whole body neutron irradiation from the 0.85 MeV Janus reactor while whole body gamma ray exposure was performed using a cobalt 60 multisource irradiator.

The original investigations focused on the life shortening effects of radiation, tumorigenesis and pathology induction, and hematological progress and late effects. Ongoing research has characterized male versus female differences in radiation response, tissue specific effects, and has applied molecular biology techniques to preserved normal tissues and tumor samples from irradiated and sham irradiated control animals (Table 1.).

An excellent synopsis of the program's experimental treatment, background, and conditions can be found in the Janus Program Report developed for the DOE (Grahn et al., 1994). The main findings of the Janus experiments are listed in Table 1 and elaborated on in the sections below.

The JM data and tissues are available to the public through Janus archival effort at Northwestern (janus.northwestern.edu). This archive contains searchable pathological, treatment, and demographic information, scans of autopsy reports, and a form to request paraffin embedded tissues. Pathological reports were prepared for 32,000 mice and histopathological data was collected for 19,000 (Grahn et al., 1992). Many tissues were preserved in paraffin, including the lung, liver, spleen, kidney, heart, and any tissues with gross lesions.

Findings on neutron protraction

JM-2 was the first and largest of the JM studies involving 11,000 mice. It sought to establish lifespan effects of neutron irradiation which was uniquely available from the Janus reactor. The JM-2 component controlled for total dose and length of treatment but varied the fractionation patterns of neutron irradiation. Surprisingly, fractionated neutron doses were more effective in life shortening than acute doses. For example, a single acute dose of 240 cGy neutron irradiation shortened the lifespan of treated mice by an average of 235 days, whereas that same dose split into 24 fractions shortened the lifespan of treated mice by 335 days, a full 100 days more. This observation was directly opposed to the trend observed following gamma ray exposure, which, when fractionated or protracted had less impact on lifespan (Ainsworth et al., 1973; Thomson et al., 1983).

JM-12 built on the results of JM-2. Using a similar study design, JM-12 sought to characterize the cause of death spectrum following different fractionation patterns of neutron irradiation. Distinct pathological spectrums were observed for each fractionated regimen. Neutron irradiation significantly increased the frequency of thymic lymphomas. Fractionation increased their frequency even more. Lymphoreticular tumors had an earlier onset, but their overall frequency went down as the lifespan of the mouse decreased. In the case of lung tumors, neutron irradiation did not even change the average age of onset, suggesting that lung tumorigenesis was somehow unaffected by neutron irradiation (Thomson et al., 1985).

Comparison between rodent species

JM-10 compared reactions of the B6CF1 strain of *Mus musculus* to *Peromyscus leucopus*. The study aimed to determine how consistent the tumorigenic and life-shortening effects of various irradiation treatments were between species of similar sizes.

Peromyscus leucopus or White-footed field deer mice belong to the Cricetidae sub-family which diverged from the *Mus musculus* lineage nearly 25 million years ago. By comparison the *Mus musculus* and *Rattus rattus* lineages diverged only 10 million years ago (Steppan et al., 2004). *Peromyscus leucopus* live nearly 50% longer than B6CF1 mice (1,450 days on average) and their aging related pathological spectra differ from B6CF1 mice. In control populations *Peromyscus leucopus* suffer from much lower rates of fatal tumorigenesis (36% vs. 84%). They were chosen as a control species to B6CF1 to follow up on previous experiments performed by George Sacher in an effort to understand the differences of radiation responses in inbred mice compared to wild rodents (Sacher et al., 1978).

In JM-10, males of each species were exposed to gamma and neutron doses delivered acutely or in 24 once-weekly doses. The results revealed that low dose irradiation caused a similar reduction in lifespan in *Peromyscus leucopus* and B6CF1 mice when measured by

percentage of total lifespan. Also, despite the differences in baseline pathological spectra, the excess mortality in *Peromyscus leucopus* attributable to irradiation was largely due to an increased incidence of neoplastic disease. However, unlike BC6F1 mice, there were no detectable differences in the effect of protracted neutron irradiation vs. acute doses. This important distinction complicated the model of neutron radiation effects even further (Thomson et al., 1986).

Low dose rate gamma irradiation

JM-4L1 and JM-4L2 extended the study of fractionation and protraction effects of gamma rays by delivering protracted gamma irradiation at extremely low dose rates while keeping the total doses and length of exposure similar to other studies. To minimize dose rates, mice were treated with $0.00136 - 0.01264 \text{ cGy min}^{-1}$ of continuous gamma ray irradiation (22 hour day⁻¹ 5 days a week) for either 23 or 59 weeks. Interestingly, for a given dose rate 59 weeks of exposure shortened the average lifespan only marginally, by 25% more than 23 weeks of exposure. Despite receiving more than twice the total dose, mice exposed for 59 weeks lost 4.5 days for each cGy delivered per week, while mice exposed for 23 weeks lost 3.6 days for each cGy delivered. Similar to the findings of prior fractionated and acute irradiation studies, most of the life-shortening impact of irradiation was attributable to increased tumorigenesis.

However, the reliability of these results from JM-4L1 and JM-4L2 are somewhat questionable due to unexpected results in the control mouse groups. The average lifespan of both control groups was unusually short compared to normal B6CF1 mice whose average lifespan is about 1,000 days. Those mice that were sham irradiated 24 times over 23 weeks lived only 850 days, while the 59 weeks sham irradiated control mice lived only 800 days, the shortest value observed in any study. Both the short life-spans and the lifespan differences between these control mice make the low-dose gamma experimental results difficult to interpret (Thomson and Grahn, 1989).

RBE of neutron vs. gamma irradiation

JM-8 and JM-9 explored the RBE of neutron vs. gamma irradiation and found that RBE increased as the total dose lowered. For example, following long term irradiation the RBE was 12 at $2.67 \text{ cGy week}^{-1}$ and 21 at $0.67 \text{ cGy week}^{-1}$ (Thomson et al., 1981a, 1981b). At acute doses of 10 cGy or lower for neutron irradiations the RBE climbed as high as 25 to 40 while at doses higher than 40 cGy it dropped to 5 (Thomson et al., 1983). RBE was not affected by the age of exposure or the choice of endpoints other than lifespan. However, both forms of radiation had less effect on lifespan when the exposed animals were older. The lifespan shortening effects for mice irradiated at 500 day of age were half of that observed for mice irradiated at 100 days.

Radioprotectors

The last of the JM studies, JM-14 explored the potential radioprotective properties of two phosphorothioate compounds, WR2721 (Amifostine) and WR151327. Each compound was designed to be injected intraperitoneally 30 minutes prior to irradiation. In the first study WR2712 was tested on 110 day old Female C57BL/6JANL x BALB/cJANL F1 mice. These mice were exposed to 206 cGy or 417 cGy of gamma irradiation 30 minutes after receiving 400 mg kg^{-1} injections of radioprotector WR2721. The injection of Amifostine protected against radiation-induced malignancies following the 206 cGy dose, especially lymphoreticular tumors. Radioprotector treated mice survived 65 days longer on average than irradiated non-treated animals. The response pattern, lifespan and pathology spectrum of mice irradiated with 417 cGy following radioprotector injection closely matched that of

mice irradiated with 206cGy, roughly corresponding to a dose reduction factor of two (Grdina et al., 1991a).

In the second part of JM-14 study, researchers attempted to assess the radioprotective properties of WR-151327 and WR2721 against neutron irradiation. While many studies had been conducted on radioprotection from gamma irradiation, this study was one of only a handful that had been conducted on neutron irradiation. In the study, male and female B6CF1 mice at 110 days old were intraperitoneally injected with 580 mg kg⁻¹ of WR-151327 or 400 mg kg⁻¹ of WR2721 30 minutes prior to receiving 10 or 40 cGy of neutron irradiation. Radiation induced tumorigenesis was reduced in injected mice and WR2721 prevented the life-shortening effects of 10cGy of neutron irradiation (Grdina et al., 1991a, 1991b).

Taken together, these studies provided a promising line of inquiry for protection from gamma ray and fission spectrum neutron irradiation.

Recent Work with the Janus Mouse and Beagle Dog Tissue Archives

The radiobiology mega-studies detailed above have remained active areas of continued research thanks to an ongoing archival effort. The volume of experimental animals and the quality of records generated has facilitated new statistical work, while the volume of well annotated paraffin embedded tissues has enabled the application of molecular techniques to the tissues.

Ongoing statistical efforts

Recent statistical efforts have revealed new gender specific responses, tissue specific radiation effects, inter-species correlations, and a broadening understanding of tumorigenesis and normal tissue responses to irradiation in the Janus Tissues.

The original findings of the Janus studies generally reported homogeneous gender effects and few tissue specific responses to neutron and gamma irradiation. However, recent analyses have shown gender, tissue, and dose specific effects. In 2006 Heidenreich et al. analyzed lung cancer incidence in mice from the Janus studies. They found gender-specific differences in lung cancer rates following gamma irradiation which was higher in females (Heidenreich et al., 2006). In 2008 Paunesku et al. analyzed the tissue specific efficacy of radioprotectors used in the JM-14 study. While only Amifostine was tested with gamma ray irradiation, both phosphorothioates were used in conjunction with neutron irradiation. In every case radioprotectors conferred protection against several neoplastic and nonneoplastic toxicities. For example, Amifostine protected mice against increased frequency of vascular tumors associated with gamma radiation and against increased frequency of liver tumors associated with the 10cGy neutron irradiation. On the other hand, few toxicities such as connective tissue tumors became more frequent following radioprotector treatment presumably because the mice lived longer which allowed for development of these “old age” tissue toxicities (Paunesku et al., 2008). Unpublished work in the Woloschak laboratory used logistic regression analysis to find gender, tissue, and dose rate specific effects in the fractionated gamma ray irradiation JM-13 studies. Controlling for the age of death, the analysis revealed that male mice developed ascites significantly more frequently in response to irradiation than females. The incidence of multiple organ system failures, toxicities in multiple organ systems, was significantly higher for females across the range treatments. Individual tumor frequencies increased following various doses of neutron irradiation for many organs, most notably in the adrenal gland, harderian gland, and kidney, all of which responded significantly to irradiation with dose rates equal to or above 0.007 cGy min⁻¹. A

similar analysis focusing on entire organ systems revealed that renal organs toxicities occur at significantly higher rates in mice irradiated at dose rates higher than $0.012 \text{ cGy min}^{-1}$.

While the original JM studies characterized the pathological spectrum following each treatment, these results were restricted to individual studies. In 1992 Carnes et al. summarized the tumor induction results of the composite JM studies. They found that 85% of mice developed neoplastic diseases. The most common tumors originated in lymph nodes (45 - 60%), the vasculature (20%), and the pulmonary system (35 - 50%). They showed neutron irradiation to have a 2 to 20 fold RBE compared to gamma irradiation for tumor induction across the range of doses tested. Like other studies, they confirmed that fractionation increased the RBE of neutron irradiation as compared to gamma irradiation (Grahn et al., 1992).

In 1998 Carnes performed a comparison of the survivorship curves of mice and dogs in response to gamma irradiation. This analysis showed that the shape of the radiation survivorship curve adjusted for differences in lifespan was similar for both mice and dogs, implying that animal size was not a significant factor in the effect of exposure on lifespan at $3.75 \text{ cGy day}^{-1}$ (Carnes et al., 1998).

Application of molecular biology to preserved tissues

Since the time when Janus experiments were initially conducted a plethora of new techniques such as microarrays, quantitative real time PCR, X-ray fluorescence microscopy (XFM), etc. became available that permit new analyses and acquisition of new information from these old tissues.

The paraffin embedded tissues from the Janus Mouse and Beagle Dog archives can still be used for identifications of protein and short nucleic acid fragment analysis. In several studies Woloschak et al. revealed gene specific mutation profiles by applying PCR coupled with Southern blot techniques to investigate the rates of mutations in lung tissues and lung adenocarcinomas in mice treated either acutely or with 24 or 60 once weekly exposures for a total dose of approximately 500 cGy gamma-rays or 50 cGy neutrons. Naturally occurring adenocarcinomas were found to have significantly higher rates of retinoblastoma protein (Rb) deletions (26%) than control tissues (3%) while radiation induced adenocarcinomas (both gamma and neutron) had the same rate of Rb deletions as healthy irradiated lung tissues (Churchill et al., 1994; Zhang and Woloschak, 1997). In the same tissues the rates of K-ras codon 12 point mutations were generally higher in adenocarcinoma but also in all tissues (healthy and neoplastic alike) exposed to neutron irradiation. (Churchill et al., 1994) Another, more recent study in the Woloschak laboratory (in preparation) employed quantitative real time PCR to compare nuclear and mitochondrial gene copy number changes across a range of doses in animals from different JM experiments. Finally, the Ya laboratory (unpublished results) also investigated microRNAs in contemporary and archived irradiated tissues from mice treated with different doses and dose rates of radiation.

Decades after embedding these tissues, tissue sections can still be used for a variety of immunohistochemistry techniques and various histological stains. Figure 2 shows the results of Mason's trichrome staining of mouse spleen samples from animals acutely irradiated with different doses (5.5 v.s. 2 Gy) of total body gamma ray irradiation. Long term effects of irradiation include fibrotic changes in tissues, and increasing doses of irradiation lead to more abundant tissue injury. Here, blue color indicates presence of collagen; therefore interspersed fibrotic collagen accumulation leads to an overall blue hue in large areas of spleen sample from a mouse exposed to 5.5Gy, and a more limited blue staining in spleen from a mouse exposed to 2Gy. Specialized staining protocols, such as Masson's trichrome

that were not used by the scientists performing initial archival studies can now be used to identify pathological structures that were previously difficult or impossible to identify.

The preserved tissues can also be analyzed for elemental and metal ions distributions. Figure 3 shows an example of X-ray fluorescence microscopy (XFM) data obtained from a tissue section of a beagle dog prostate diagnosed with hyperplasia and chronic prostatitis. In this tissue section, as shown by the hematoxylin & eosin stain (lower right), normal prostate tissue morphology predominates, however, with an apparent absence of normal prostate morphology in the lower right area of the sample. This same tissue region (lower right) has a decreased zinc concentration as established by XFM analysis. Healthy prostate tissue is characterized by abundant presence of zinc, while prostate tumors and nonfunctional tissue no longer contain much zinc as the normal function of the tissue is lost (Costello and Franklin, 1998). Sulfur signal by comparison, indicates density of the tissue, since local concentration of this element can best be associated with protein content. Locally high iron intent, depending on the distribution pattern, can be a result of hemoglobin accumulation (presence of blood vessels) or indicate functional changes in the tissue. In this prostate tissue section, XFM Sulfur signal maximum corresponds to 1.33 micrograms per centimeter square of the sample, iron signal maximum is 0.119 $\mu\text{g}/\text{cm}^2$, while zinc signal maximum is 0.0334 $\mu\text{g}/\text{cm}^2$. Evidently, even after 30+ years these tissues are in adequate condition to permit XFM studies on metal content in the tissues, a method which was not available at the time the experiments were initially performed.

Conclusions

The original and ongoing work on the samples from Argonne National Laboratory external beam studies have helped to reveal the basic model of gamma and neutron irradiation in beagle dogs, mice and, by extension, humans. While the original work developed the RBE models for lifespan and tumorigenesis under a range of radiation qualities and exposure patterns, the ongoing work has begun to reveal new tissue, gender, dose, and dose rate specific effects following neutron and gamma irradiation. It is expected that more results from additional studies of the tissues and database will continue to enrich radiation biology as the existing samples and study records are subjected to more investigation scrutiny by a body of scientists given access to these resources by archival dissemination efforts.

The openness characterizing the present approach to Argonne experimental data and tissues is mirrored in related work from other radiobiology archives hosted by the ERA (bfs.de/en/bfs/forschung/Era_pro.html) and JRA. Formal and informal exchanges abound between these organizations and the Janus archive hosted by the Woloschak laboratory. It is the hope of these archival efforts to extract more data from these expensive and irreplaceable experiments in order to reduce or obviate new large animal studies and preserve findings of researchers who participated in the studies.

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Fig 1. ^{60}Co reactor used in beagle dog experiments

Many of the beagle dog experiments involved (nearly) continual irradiation. Pictured is the reactor and kennel setup that ensured dogs were exposed to a consistent dose of radiation 22 hours a day. Organ level dose estimates were established using a dog phantom; the random movement of dogs within their kennel cages ensured an even distribution of dose.

Figure 2. Masson's trichrome staining of mouse spleen samples from animals irradiated with different doses of total body gamma ray irradiation

Long term effects of irradiation include fibrotic changes in tissues, and increasing doses of irradiation lead to more abundant tissue injury. Images shown come from spleen tissue sections from mice acutely irradiated with 5.5 Gy (a) and 2 Gy (b). These tissue sections were stained with Masson's trichrome dye; blue color indicates presence of collagen; therefore blue areas indicate either vasculature (arrows) or interspersed fibrotic collagen deposition leading to an overall blue hue in large areas of sample from a mouse exposed to 5.5Gy.

Figure 3. X-ray fluorescence microscopy of dog prostate tissue

XFM analysis can be performed on sections of paraffin embedded dog tissues to show the distributions of various elements. In this example, we imaged by XFM a section of prostate tissue from a control dog (ID # 1686): a colony control male, which died at 5613 days of age. Lower right corner image shows an H&E image, while XFM 2d maps show distribution of sulfur (S), iron (Fe) and zinc (Zn) in an adjoining tissue section. At necropsy, prostate pathology from this animal showed hyperplasia and chronic prostatitis. Upper portion of the tissue shows a more regular prostate morphology as per hematoxylin and eosin (H&E) staining. Corresponding X-ray fluorescence (XFM) images show the highest zinc concentration in the same region, while distributions of sulfur and iron follows different patterns. (Elemental distribution is presented by false colors, with highest signals indicated by yellow and red, and lowest by black and purple.)

Table 1**Main Janus Findings**

Finding	Study	Reference
Protracted neutron irradiation is more effective at promoting tumorigenesis and life shortening in mice.	JM-2	(Ainsworth et al., 1973)(Thomson, Williamson, Grahn, & Ainsworth, 1981a).
Neutron irradiation decreases the frequency of lymphoreticular tumors and lung tumors. Other tumors are more frequent.	JM-12	(Thomson, Williamson, & Grahn, 1985)
Lifespan reduction is a fixed percentage of total lifespan for a given radiation treatment between similarly sized species (<i>Mus musculus</i> and <i>Peromyscus leucopus</i>)	JM-10	(Sacher, Tyler, & Trucco, 1978).
At very low gamma irradiation dose rates, increased exposure time has a diminishing impact on life shortening.	JM-4L1 JM-4L2	(Thomson & Grahn, 1989)
The Relative Biological Effectiveness (RBE) of neutron vs. gamma irradiation is highest at low dose rates and low total doses.	JM-8 JM-9	(Thomson, Williamson, Grahn, & Ainsworth, 1981b) (Thomson, Williamson, & Grahn, 1983)
Mutation profiles vary by gene and tumor. For example spontaneous lung adenocarcinomas show high rates of mRb deletions and low rates of p53 mutations while healthy and irradiation induced adenocarcinomas display the opposite set of mutations, low mRb deletions and high rates of p53 mutations.	across studies	(Zhang & Woloschak, 1997) (Zhang & Woloschak, 1998)(Churchill, Gemmell, & Woloschak, 1994)
Radioprotectors WR2712 and WR151327 were effective at reducing tumorigenesis and the life shortening effects of gamma but not neutron irradiation. Radioprotectors protected many, though not all, tissues from non-tumor toxicities.	JM-14	(Grdina, Carnes, Grahn, & Sigdestad, 1991) (Grdina, Wright, & Carnes, 1991) (Paunesku et al., 2008)
Gamma irradiation is more effective at causing lung cancer in female mice and ascites in male mice.	Across studies	(Heidenreich, Carnes, & Paretzke, 2006). (Woloschak, unpublished)
The vast majority of irradiated mice (85%) die of neoplastic disease originating in the lymph nodes (45-60%), vasculature (20%), and pulmonary system (35-50%)	Across studies	(Grahn, Lombard, & Carnes, 1992)