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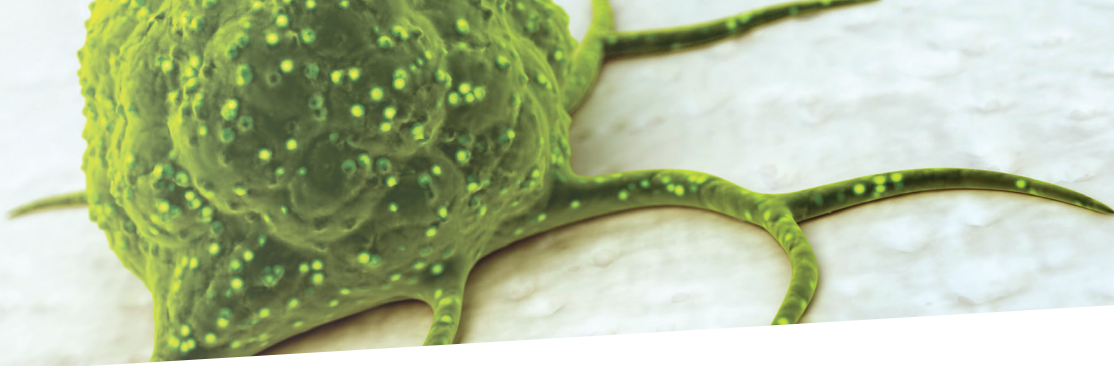


Research Models and Services
Oncology – Mutant Mice

Radiosensitivity of immunodeficient mice in oncology studies

INTRODUCTION

Mouse models are powerful *in vivo* systems that play an important role in biomedical research. In recent years, there has been growing interest in the use of immunodeficient mice for a wide variety of applications, including immunology, infectious diseases, autoimmunity, and cancer. Mice with compromised immune systems are invaluable tools that not only provide critical insight into genes essential for immune function, but when combined with the transplantation of human cells or tissues, they also have enormous potential to provide scientists with relevant models of human disease.

Numerous immunodeficient mouse strains have been developed, and they can be broadly grouped into three categories, with each strain carrying different genetic mutations and levels of immune deficiencies (Belizario, 2009). Some of the similarities and differences across a subset of these immunodeficient models are summarized in Table 1. The early generations of immunodeficient mice, which include Nude (Flanagan, 1966), Severe Combined Immunodeficiency (SCID) (Bosma,1983), Rag1$^{-/-}$ (Mombaerts, 1992), and Rag2$^{-/-}$ (Shinkai, 1992) models, harbor single genetic mutations that confer modest immune dysfunction. While Nude mice lack mature T cells, SCID and Rag1/2 mice exhibit greater immune deficiencies due to a lack of both B- and T-cells. SCID mice are also hypersensitive to radiation, while Nude and Rag1/2 models exhibit radiosensitivity comparable to wild-type animals (Shultz, 2007).

A major limitation of these relatively simple models is the presence of residual immunity, particularly a high level of natural killer (NK) cell activity. To address this limitation, strains were developed carrying multiple genetic alterations to further compromise the immune system. Perhaps the most well-known of these strains is the non-obese diabetic (NOD)-SCID mouse (Shultz, 1995). The breeding of SCID mice into the NOD model impairs (but does not completely eliminate) NK-cell activity. These animals have been invaluable to the study of hematopoiesis (Dick, 1997). The development of immunodeficient mice completely lacking NK cells was made possible with mice carrying a deletion or truncation of the common gamma chain/*Il2rg* (Cao, 1995; DiSanto,1995; Ohbo, 1996). Since *Il2rg* is required to mediate the effects of multiple cytokines including IL-2, IL-4, IL-7, IL-9, and IL-15, disruption of the *Il2rg* gene leads to major defects in lymphocyte and lymphoid tissue development. Mice carrying the *Il2rg* null allele or truncated mutant have been bred into the non-obese diabetic-severe combined immunodeficiency (NOD. SCID) model to generate the highly immunodeficient NSG™ (Shultz, 2005) and CIEA NOG mouse® (Ito, 2002) mice, respectively. An equally powerful model is the Rag2/*Il2rg* double knockout (R2G2) immunodeficient mouse (Goldman, 1998; Mazurier, 1999), a model that is now available from Inotiv. In addition to comparable defects in B-, T-, and NK-cells with NSG™/NOG mice, the presence of the *Rag2* mutation in place of *scid* has the benefit of providing a radioresistant phenotype, which is an important feature for a variety of research applications.

There has been increasing interest in the use of the new generations of highly immunodeficient mice for oncology studies. Traditional xenograft models using conventional cancer cell lines engrafted into Nude mice have long been used by researchers to test the efficacy of anti-cancer therapeutics. However, the successful translation of promising preclinical therapies to the clinic has been challenging, with 85% of early stage clinical trials ending in failure (Ledford, 2011).

This has driven the need for improved pre-clinical models that better predict patients' responses to therapeutic interventions. One approach that has garnered much attention is the development of "avatars" or patient-derived xenograft (PDX) models (Hidalgo, 2014). Unlike xenografts using immortalized cultured cell lines, PDX models are based on the transplantation of fresh tumor fragments or cell suspensions obtained directly from patients into strains of highly immunodeficient mice.

By retaining the histological and genetic characteristics of the primary tumor, these models can serve as more representative pre-clinical tools to investigate the efficacy and safety of established and novel therapeutic strategies. Indeed, this strategy has been used to test and refine treatments for specific cancer patients based on data generated from a patient-specific avatar.

Another major application of immunodeficient mice has been the development of "humanized" immune systems (Shultz, 2012). This is achieved via the transplantation of hematopoietic stem cells (HSCs) or mature immune cell lineages into newborn or adult immunodeficient mice and has been extensively used for studies of immunology and immune-related diseases.

Although there is considerable excitement regarding the use of immunodeficient mice for oncology studies, the wide variety of strains that are available can make choosing an appropriate model difficult and confusing. This is particularly relevant to researchers investigating the efficacy of radiation therapy, since Rag1/2$^{-/-}$ mice and SCID models display inherent differences in their sensitivities to radiation-induced DNA damage (Shultz, 2007).

The purpose of this white paper is to provide an overview of the advantages of the Rag2/Il2rg model with a focus on the factors that contribute to differential radiation sensitivity between the Rag and SCID models. In addition, this paper presents the results from a radiosensitivity and an immune cell profile study. Specifically, the radiosensitivity study compared the growth and survival of Inotiv's Rag2/ Il2rg (R2G2) model to the NSG™ model using three different doses of radiation, while the immune cell profile study evaluated immune cell subsets using the spleens of R2G2® and NSG™ mice. Overall, the impact of radiosensitivity on experimental design and the interpretation of the results will also be explored. With this knowledge in hand, scientists can make better informed decisions when choosing an appropriate immunodeficient model for their oncology studies.

Table 1: Immune system characteristics of a representative sample of immunodeficient mouse models

MODEL	NOMENCLATURE	HAIR	T CELLS	B CELLS	NK CELLS
NUDE MICE					
Athymic nude	Hsd:Athymic Nude-*Foxn1*^{nu}	No	Nonfunctional	Functional	Functional
NMRI nude	HsdCpb:NMRI-*Foxn1*^{nu}	No	Nonfunctional	Functional	Functional
RAG2/IL2RG DKO					
R2G2	B6;129-*Rag2*^{tm1Fwa}*Il2rg*^{tm1Rsky}/DwlHsd	Yes	Nonfunctional	Functional	Nonfunctional
Rag2/Il2rg	B10;B6-*Rag2*^{tm1Fwa}*Il2rgt*^{m1Wjl}	Yes	Nonfunctional	Functional	Nonfunctional
CIEA BRG	C.Cg-*Rag2*^{tm1Fwa}*Il2rg*^{tm1Sug}/JicTac	Yes	Nonfunctional	Functional	Nonfunctional
Rag2- γc-	C;129S4-*Rag2*$^{tm1.1Flv}$*Il2rg*$^{tm1.1Flv/J}$	Yes	Nonfunctional	Functional	Nonfunctional
SCID MICE					
C.B-17 SCID	C.B-17/ IcrHsd-*Prkdc*^{scid}	Yes	Nonfunctional	Nonfunctional	Functional
SCID/Beige	C.B-17/ IcrHsd-*Prkdc*^{scid}*Lyst*$^{bg-J}$	Yes	Nonfunctional	Nonfunctional	Impaired
NOD.SCID	NOD.CB17-*Prkdc*^{scid}/ NCrHsd	Yes	Nonfunctional	Nonfunctional	Impaired
NSG™	NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ	Yes	Nonfunctional	Nonfunctional	Nonfunctional
CIEA NOG mouse®	NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug}/JicTac	Yes	Nonfunctional	Nonfunctional	Nonfunctional
NCG	NOD-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}/NjuCrl	Yes	Nonfunctional	Nonfunctional	Nonfunctional
B-NDG	NOD.CB17-*Prkdc*^{scid} *IL2rg*^{tm1}/BcgenHsd	Yes	Nonfunctional	Nonfunctional	Nonfunctional

COMPARISON OF RAG2$^{-/-}$ AND SCID MODELS

Along with Nude models, SCID mice and their multigenic counterparts are the most commonly used immunodeficient mouse strains. First identified in a colony of C.B-17 mice (Bosma, 1983), SCID mice harbor a spontaneous mutation in the *Prkdc* gene. *Prkdc* encodes the catalytic subunit of the DNA dependent protein kinase (DNA-PK) and is required for non-homologous end joining (NHEJ). Since NHEJ is essential for V(D)J recombination, a process which gives rise to immunoglobulin and T-cell receptor diversity, the mutation of *Prkdc* results in failed B- and T-cell maturation (Belizario, 2009).

Unlike the spontaneous mutation carried by SCID mice, Rag1$^{-/-}$ and Rag2$^{-/-}$ animals were engineered to harbor germline deletions of the recombination-activating genes (Rag1 or Rag2, (Mombaerts, 1992; Shinkai, 1992). Since the enzyme products encoded by the Rag genes serve to ensure proper V(D)J recombination, their deletion also leads to B- and T-cell deficiencies. Despite similar phenotypes, Rag2$^{-/-}$ mice are more prevalent in the literature than Rag1$^{-/-}$ mice. This is perhaps due to the wider distribution of the model and availability on multiple inbred genetic backgrounds. Notably, the Rag2$^{-/-}$ strain serves as the breeding partner for Il2rg$^{-/-}$ mice to create the Rag2/Il2rg double knockout.

Although SCID and Rag2$^{-/-}$ mice are often described as exhibiting similar immune dysfunction, the level of B- and T-cell function differs between the two models. Indeed, SCID mice are known to become "leaky" with age, such that virtually all mice more than 1-year old contain detectable levels of B and T lymphocytes (Nonoyama, 1993), which can inhibit xenograft growth. In contrast, Rag2$^{-/-}$ mice do not display a "leaky" phenotype and are thus considered more immunodeficient than SCID mice. This feature of Rag2$^{-/-}$ mice also makes them better suited for longterm *in vivo* assays.

Despite some obvious advantages of the Rag2$^{-/-}$ model over the SCID model, the SCID model remains more heavily used by researchers. One potential reason for this discrepancy is the fact that the SCID strain was identified in 1983, almost a full decade before advances in recombinant DNA technology led to the generation of Rag2$^{-/-}$ animals. During this time, SCID mice were made widely available to researchers around the world who, in turn, generated reliable data regarding the immunological properties of the model and optimization of protocols for human cell transplantation. Nevertheless, as the interest and use of immunodeficient mice continues to rise, scientists are becoming more aware of the advantages offered by other models, including Rag2$^{-/-}$ mice.

RADIOSENSITIVITIES OF RAG2$^{-/-}$ AND SCID MODELS

In addition to having a "leaky" immunodeficient phenotype, the SCID mouse is known for being hypersensitive to ionizing radiation (Biedermann, 1991; Fulop, 1990). As noted earlier, NHEJ plays multiple roles in cells. It is required for V(D)J recombination, as well as for proper DNA repair. Consequently, the disruption of NHEJ through the mutation of the *Prkdc* gene renders SCID mice sensitive to DNA damage induced by radiation exposure. Furthermore, as the mutation is present in every cell, all tissues have the potential to be affected. In contrast, the radiosensitivity of Rag1/2 mice is comparable to wild-type mice, as the Rag1/2 enzymes are only required for V(D) J recombination. Several studies have tested the radiosensitivity of immunodeficient mouse strains (e.g. Biedermann, 1991; Fulop, 1990; Goldman, 1998; Shultz, 2000; Shultz, 2005). Overall, the data support the view that Rag1/2 mice are more radioresistant than SCID mouse strains, and help to establish the advantage of the Rag2/Il2rg model for studies requiring radiation, especially those studies that may require higher doses of radiation exposure.

There are two main types of studies in which the radiosensitivities of immunodeficient mice require careful consideration. First, whole-body irradiation is often carried out as a pre-conditioning myeloablative step to enhance the engraftment of transplanted hematopoietic cells (e.g., Goldman, 1998). For example, this is a critical step prior to generating "humanized" immune system mice. Sub-lethal irradiation has been shown to stimulate the bone marrow production of stem cell factor (SCF) production and facilitate hematopoiesis (Broudy, 1997). Irradiation also serves to eliminate endogenous HSCs and other residual immune cells to make room for engrafted cells. Notably, the higher the radiation dose, the greater the likelihood of achieving more complete myeloablation. For instance, Down et al. used murine bone marrow chimera models to evaluate the efficacy of host total body irradiation given at different doses for engraftment of syngeneic and allogeneic bone marrow. While partial engraftment of syngeneic marrow was seen at single doses as low as 2Gy, the donor component became increasingly more prominent with increasing radiation dose (100% was achieved at 7Gy). Importantly, resistance of the host appeared to prevent allogeneic engraftment until 5.5Gy, and the authors noted that there was a steep radiation dose response observed after that level, so that the level of chimerism ≥6Gy became comparable with syngeneic engraftment (Down, 1991). Of course, investigators must balance the benefits of achieving higher myeloablation with the toxic side effects of radiation by considering factors such as the strain and age of recipient mice.

Second, the radiosensitivities of immunodeficient mice are also an important factor to consider for oncology studies. Along with chemotherapy, radiation therapy remains an integral part of anti-cancer treatment regimens with up to 75% of patients requiring some form of radiation during the course of treatment (Kahn, 2012). In addition to the direct effects of radiation on the tumor, there are also bystander effects (also known as abscopal effects) induced by radiation (mostly at higher doses). These effects are thought to be immunemediated and their implications for cancer therapy are only beginning to be unraveled (Sologuren, 2014; Demaria, 2004). On this note, in a study by Shiraishi et al., it was shown that the administration of a chemokine after local tumor site irradiation (using a dose of 6 Gy) prolonged survival of the mice. Indeed, the tumor was completely eradicated in about 50% of the animals with daily administration of the chemokine, and importantly, tumor growth at the non-irradiated site was inhibited, suggesting the chemokine enhanced the abscopal effect of radiation (Shiraishi, 2008). While mouse-based radiation therapy studies certainly exist in the literature (e.g., Speers, 2016; Karnak, 2014; Shiraishi, 2008), advances in technology, such as small animal image-guided radiation platforms (Butterworth, 2015), will undoubtedly contribute to a rise in the use of radiation therapy in pre-clinical studies in the near future. The availability of radioresistant mice including the R2G2 model will allow scientists to implement these advanced technologies into their research.

In addition to radiation, the mechanism of action of some conventional anti-cancer chemotherapeutics, such alkylating and alkylating-like agents (e.g., cyclophosphamide and cisplatin), is to induce DNA damage (Cheung-Ong, 2013). Consequently, SCID mice have been shown to have increased sensitivity to DNA-damaging cancer chemotherapy agents. The benefits of utilizing radioresistant immunodeficient mice for oncology studies is highlighted by a study from Bertilaccio and colleagues, who reported 100% engraftment of human chronic lymphocytic leukemia cells (CLL) into Rag2/Il2rg mice but observed no growth in Nude mice (Bertilaccio, 2010). Further, the use of Rag2/Il2rg mice allowed Bertilaccio et al. to test the efficacy of standard-of-care treatments for CLL, including cyclophosphamide. Given that cyclophosphamide is a DNA-damaging agent, SCID models may have had limited utility for this type of validation experiment due to their enhanced sensitivities to radiation and chemotherapy, relative to the Rag2/Il2rg model.

Due to the limitations associated with SCID mice, researchers frequently utilize Nude mice for their oncology studies (Willey, 2015). The benefits of Nude mice include a lack of hair, which allows for imaging, and radioresistance for radiation therapy studies. While this strain is sufficient when using conventional cell lines, the presence of residual immunity including B cells and high NK-cell activity limits the usefulness of this strain in the development of superior PDX models.

To overcome this hurdle, some researchers have employed the use of multiple immunodeficient mouse strains in an effort to generate tumor models that can be used with radiation. For example, in a recent report, the therapeutic potential of a novel combination antibody therapy (Pan-HER) augmented with radiation therapy was tested against a PDX model of head and neck squamous cell carcinoma (HNSCC) (Francis, 2016). To generate this model in a radioresistant immunodeficient model, the researchers passaged fresh primary tumor fragments in SCID mice before transferring the graft onto Nude mice (supplied by Inotiv). Although the results of this pre-clinical study were encouraging, it is unclear as to what extent the PDX may have changed during the passage from one strain of immunodeficient mice to another. The propagation of tumor tissue using this method may permit the selection of more aggressive cells that have acquired the ability to grow in the less immunodeficient Nude strain. In addition, whether the presence of residual immune function in Nude mice positively or negatively influenced the observed therapeutic response is unknown. While there is evidence that PDX tumor models remain relatively stable at the histological and genetic levels (Willey, 2015), this has yet to be examined when passaging across multiple immunodeficient mouse strains. Of course, one alternative approach is to use a single immunodeficient model, such as Rag2/Il2rg, to establish the xenograft in a radioresistant model and minimize the risk of cellular genetic drift over serial passages. In addition to the potential for superior data generation, this approach would also help save valuable time and resources.

Overall, researchers need to carefully consider immunodeficient model selection for their studies. This can be a complex decision, as many factors are involved and many strains of immunodeficient mice are available. When a model with enhanced radioresistance is warranted, it is advisable to choose a Rag-based model, such as the highly immunodeficient R2G2, which is a suitable host for xenografts using conventional immortalized cancer cell lines and patient-derived tissues or cells, and is also ideal for studies examining the efficacy of radiation and DNA-damaging agents as part of a cancer treatment regimen.

In the following section, the results of a radiosensitivity and immune cell profile study are presented for Inotiv's R2G2 model in comparison to the NSG™ mouse model.

CHARACTERIZATION OF INOTIV'S RAG2/IL2RG (R2G2) MODEL

A set of studies were conducted using Inotiv's Rag2/Il2rg (R2G2) model to empirically characterize its radiosensitivity and immune cell profile. For comparative purposes, NSG™ NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) mice were also included, and this model has been shown to tolerate radiation doses of up to 4Gy (Shultz, 2005) and its immune cell profile is well characterized (Table 1). A summary of the methods and results of the study are presented below.

MATERIALS AND METHODS

Radiosensitivity study

On Study Day 1, groups of R2G2 and NSG™ mice (n=5 per group) aged 7-8 weeks were exposed to one of three doses of whole body irradiation: 2Gy, 4Gy or 6Gy. Mice were irradiated using a RadSource RS-2000, which employs a 160 kV, 4.2 kW x-ray source. After receiving the radiation dose, animals were monitored daily for changes in body weight and survival for up to 29 days. Animals were euthanized when moribund (e.g. >20% body weight loss, loss of righting reflex). All animals were maintained on Teklad Global Rodent Diet 2918 (18% protein).

Flow cytometric analysis of immune cells

Spleens were harvested from each animal, and two spleens were pooled to make a single sample for flow cytometric analysis. Analysis of spleen cells was performed on an Invitrogen™ Attune™ flow cytometer. A cohort of 20 R2G2 and 10 NSG (females only) (The Jackson Laboratory), aged 7-8 weeks, was run on a MI-TAM panel and a custom lymphoid panel (Molecular Bioscience, Inc). The MI-TAM panel included antimouse markers CD11b (pan-myeloid lineage marker), F4/80 (pan-macrophage), Ly-6C (myeloid derived suppressor cell exclusion), MHC Class II (M1 marker), CD45 (pan-hematopoietic marker), CD206 (M2), Ly-6G (Myeloid-derived suppressor cell exclusion), CD11c (dendritic marker), CD3/CD19 (T and B exclusion). The custom lymphoid panel included anti-mouse markers CD3 (total T), CD4 (T helper), CD8 (cytotoxic T), CD 19 (B cells), CD45 (pan-hematopietic) and CD49b/CD335 (NK cells).

RESULTS

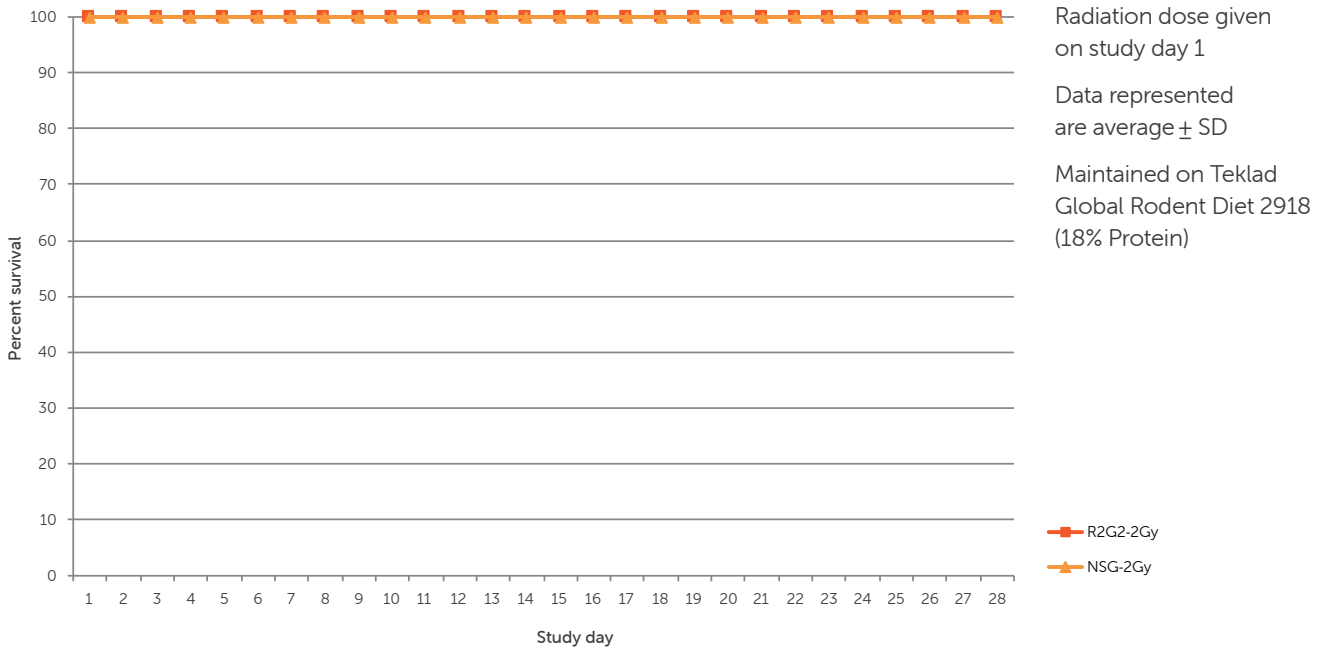
Radiosensitivity study

The effect of varying doses of radiation on the survival and body weight of R2G2 and NSG™ mice was evaluated. Groups of mice were irradiated with radiation doses of 2Gy, 4Gy, or 6Gy and monitored for 29 days. As shown in Figures 1A and 2A, no meaningful differences in survival or body weight change were observed between the R2G2 and NSG™ models at the lowest dose of radiation (2Gy). Survivability was 100% for both models at 29 days (Figure 1A), and after an initial small drop in body weight in both models, after 29 days the R2G2 and NSG™ mice had gained an average of 5.1% and 8.8%, respectively (Figure 2A). In contrast, at a radiation dose of 4Gy, NSG™ mice experienced 100% mortality by post-irradiation day 8 (Figure 1B), and showed a drastic loss of body weight (Figure 2B). Meanwhile, R2G2 mice displayed 100% survivability (Figure 1B), and robust body weight gain showing a similar trend to that of the 2Gy radiation dose (Figure 2B). At the highest radiation dose of 6Gy, approximately 50% of the R2G2 mice survived for 14 days, but by the 17-day timepoint, there was 100% mortality (Figure 1C). For the NSG™ mice, these animals experienced 100% mortality by the 5-day timepoint (Figure 1C). Thus, with regard to survivability, the R2G2 mice survived approximately three-fold longer than the NSG™ mice following a radiation dose of 6Gy. As for body weight, both models exhibited reduced body weight after the 6Gy dose of radiation, albeit the body weight loss of the R2G2 mice was gradual, in contrast to the rapid body weight loss seen in the NSG™ mice (Figure 2C).

Overall, the results of the radiosensitivity study demonstrate that R2G2 mice are less radiosensitive at radiation doses of 4Gy and 6Gy, relative to NSG™ mice. Indeed, even at the highest dose of radiation tested (6Gy), R2G2 mice showed 50% survivability at 14 days, and only a gradual loss of body weight over the time-period leading up to 100% mortality of these animals. This contrasts with the 100% mortality observed for NSG™ mice after only 5 days, which was accompanied by dramatic body weight loss.

FIGURE 1. PERCENT SURVIVAL OF INOTIV'S R2G2 MODEL AND NSG™ MICE IRRADIATED AT 2GY (A), 4GY (B), AND 6GY (C) (GREEN = R2G2; GREY = NSG™)

Figure 1A: Percent Survival of Inotiv's R2G2 model and NSG™ mice irradiated at 2Gy

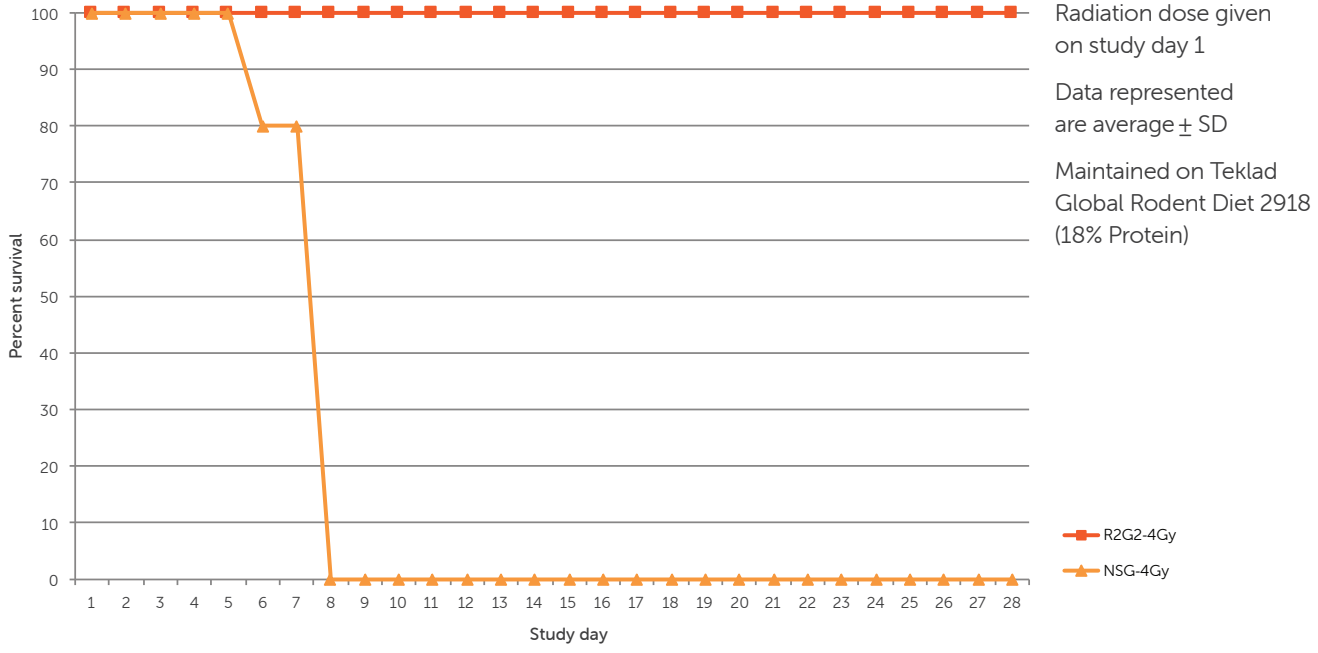


Radiation dose given on study day 1

Data represented are average ± SD

Maintained on Teklad Global Rodent Diet 2918 (18% Protein)

Figure 1B: Percent Survival of Inotiv's R2G2 model and NSG™ mice irradiated at 4Gy

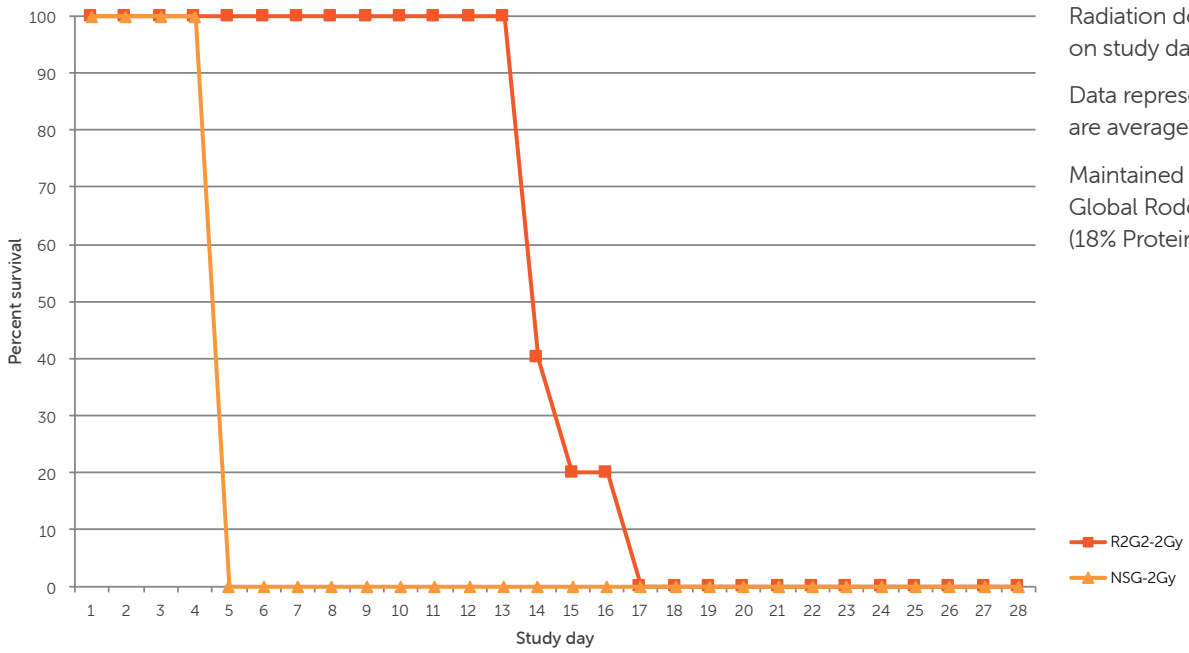


Radiation dose given on study day 1

Data represented are average ± SD

Maintained on Teklad Global Rodent Diet 2918 (18% Protein)

Figure 1C: Percent Survival of Envigo's R2G2 model and NSG™ mice irradiated at 6Gy



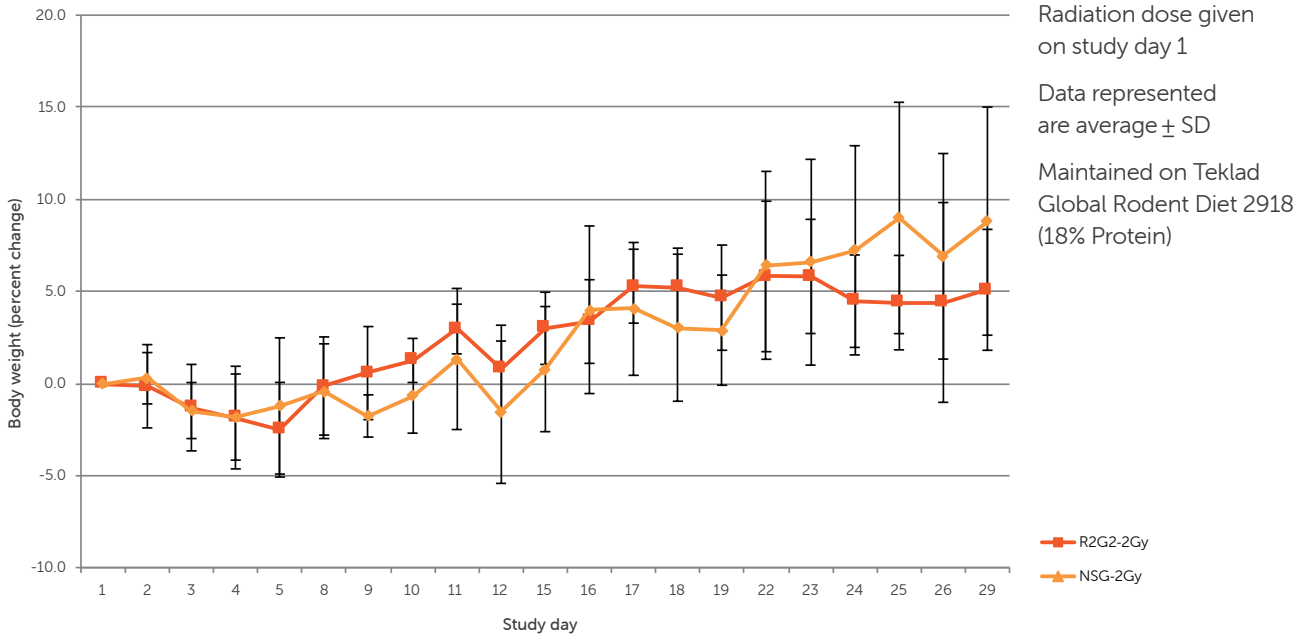
Radiation dose given on study day 1

Data represented are average ± SD

Maintained on Teklad Global Rodent Diet 2918 (18% Protein)

FIGURE 2. PERCENT CHANGE IN BODY WEIGHT OF INOTIV'S R2G2 MODEL AND NSG™ MICE IRRADIATED AT 2GY (A), 4GY (B), AND 6GY (C) (GREEN = R2G2; GREY = NSG™)

Figure 2A: Percent change in body weight of Inotiv's R2G2 model and NSG™ mice irradiated at 2Gy

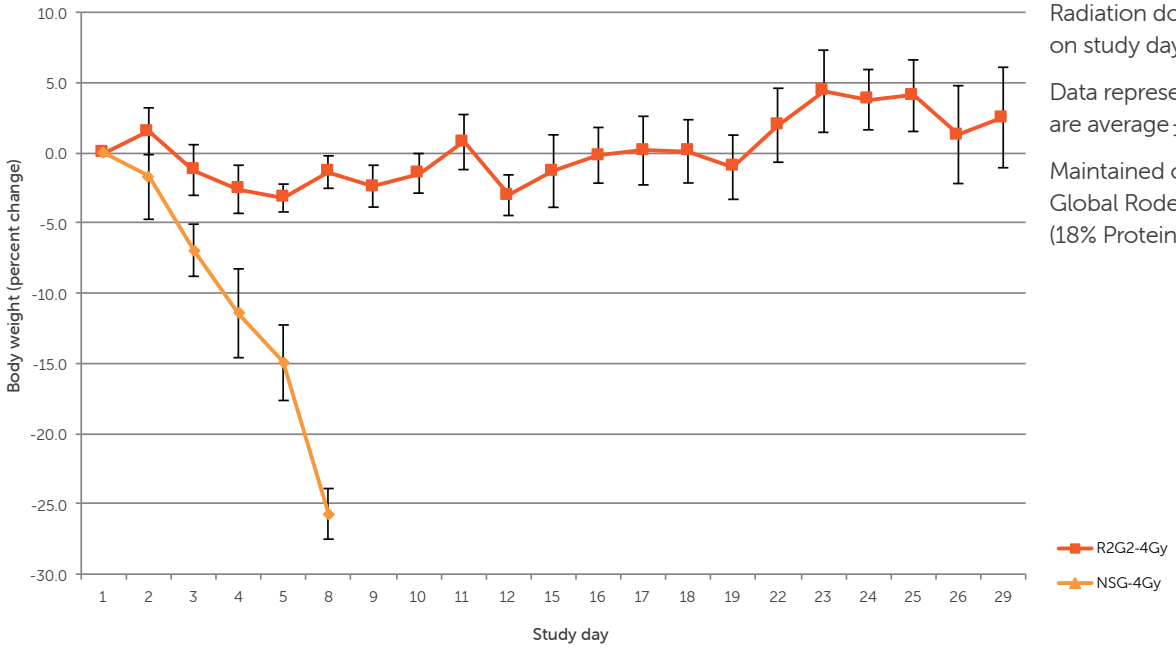


Radiation dose given on study day 1

Data represented are average ± SD

Maintained on Teklad Global Rodent Diet 2918 (18% Protein)

Figure 2B: Percent change in body weight of Inotiv's R2G2 model and NSG™ mice irradiated at 4Gy

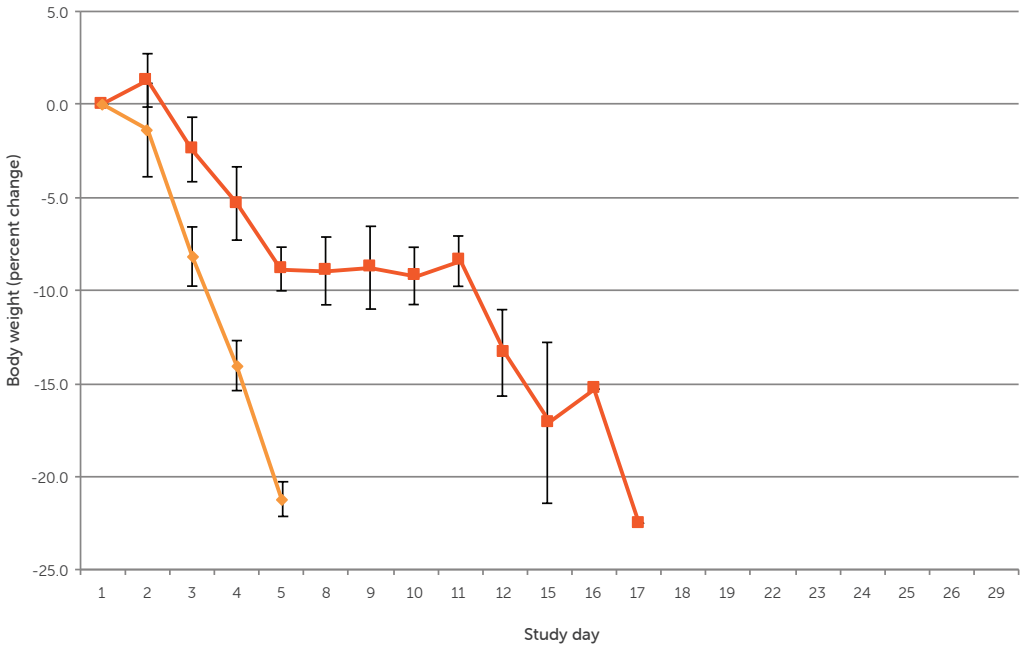


Radiation dose given on study day 1

Data represented are average ± SD

Maintained on Teklad Global Rodent Diet 2918 (18% Protein)

Figure 2C: Percent change in body weight of Inotiv's R2G2 model and NSG™ mice irradiated at 6Gy



Radiation dose given on study day 1

Data represented are average ± SD

Maintained on Teklad Global Rodent Diet 2918 (18% Protein)

Table 2: Summary of flow cytometric data for immune cell subsets in R2G2 and NSG™ mice

MODEL			FEMALE NSG (N=3)	FEMALE R2G2 (N=8)	MALE R2G2 (N=9)
T Cells	CD3+	% CD45 +	1.4 ± 1.1	1.1 ± 1.0	1.0 ± 0.9
T Helper Cells	CD4+	% CD3 +	9.9 ± 0.8	4.7 ± 3.1*	5.5 ± 3.7
T Cytotoxic Cells	CD8+	% CD3 +	1.9 ± 0.8	1.7 ± 0.6*	1.6 ± 1.5
B Cells	B220+/Ly6c+	% CD45 +	5 ± 2.2	2.2 ± 1.1*	2.6 ± 1*
NK Cells	CD335+/CD49b+	% CD45 +	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
Dendritic Cells	MHCII+/CD11c+	% CD45 +	2.7 ± 0.9	0.9 ± 0.7*	1.2 ± 0.9*
Macrophages	F4/80+	% CD11b +	12.1 ± 1.3	2.6 ± 0.9*	3.6 ± 0.6*
M1 Macrophages	MHCII+	% CD11b +	0 ± 0	0.6 ± 0.2*	1.0 ± 0.2*
M2 Macrophages	CD206+	% CD11b +	3.2 ± 0.5	1.1 ± 0.3*	1.6 ± 0.4*
G-MDSC	Ly-6C+	% CD11b +	60.2 ± 7.8	77.1 ± 10.3*	70.7 ± 10.8
M-MDSC	Ly-6C+	% CD11b +	18.3 ± 2.6	17.0 ±7.6	22.1 ± 8.5

* Significant difference from Female NSG, P<0.05

Immune cell profile study

Flow cytometric analysis was conducted to evaluate immune cell subsets using the spleens of R2G2 and NSG™ mice. This analysis confirmed that both the R2G2 and NSG™ models are profoundly immunodeficient, and the two models are comparable in regards to the percentage of CD3+ T cells, CD8+ cytotoxic T cells, and NK cells (Table 2). Notably, both male and female R2G2 mice have significantly lower levels of B cells, dendritic cells, and total macrophages as compared to NSG™ mice (Table 2). Furthermore, R2G2 mice have lower levels of CD4+ T helper cells, and this was statistically significant in the R2G2 females relative to the NSG™ (female) mice (Table 2). M1 and M2 macrophage levels were also examined, and it was found that M1 macrophage levels were higher in R2G2 mice, while M2 macrophages were lower in the R2G2 mice (Table 2). In addition, myeloid derived suppressor cells (MDSCs) were examined by subtype, specifically, granulocyte-like MDSCs (G-MDSC) and monocyte-like MDSCs (M-MDSC). This analysis revealed that R2G2 mice have more G-MDSCs than NSG™ mice, while both models had comparable levels of M-MDSCs (Table 2).

Taken together, the results of this comparative study demonstrate that R2G2 mice are less radiosensitive than NSG™ mice at radiation doses of 4Gy and 6Gy. Further, the flow cytometric analysis of immune cells shows a comparable degree of immunodeficiency between these two models.

CONCLUSIONS

The development of highly immunodeficient strains of mice has ushered in an era of advanced human cancer models. In addition to providing a more relevant platform for drug testing, these models also hold considerable promise for experiments involving radiation therapy. However, as touched upon in this paper, not all immunodeficient mice are equal, and understanding the inherent differences associated with each strain is critical when planning a study. The results of an internal study comparing the radiosensitivity and immune cells profiles of Inotiv's R2G2 mice and NSG™ mice, confirm that R2G2 mice are less radiosensitive (at 4Gy and 6Gy) than NSG™ mice, while both are highly immunodeficient. Thus, the R2G2 mouse features a higher degree of immunodeficiency than Nude mice, and greater radioresistance than SCID strains. This makes the R2G2 mouse an ideal model not only because of its high degree of immunodeficiency, but also for carrying out pre-clinical studies involving radiation therapy or pretreatment, and novel DNA-damaging chemotherapeutic agents. Thus, for scientists pursuing studies that require an immunodeficient model and exposure to higher doses of irradiation, such as humanization and tumor radiation therapy protocols, the R2G2 model is the superior choice.

A wide variety of options are available when it comes to choosing an immunodeficient mouse, and thus model selection is not always straightforward. Discussions with Inotiv employees when planning a project can help save time, effort, and resources.

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