Utility of the highly immunodeficient SRG rat for combined drug efficacy, pharmacokinetics, and toxicology studies in tumor-bearing animals

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Introduction

Immunodeficient rodent models are vital preclinical models, allowing xenografting of human cells and tissues for drug efficacy and tolerability testing in a human-like disease setting. Historically, immunodeficient mice have been the standard species for cancer xenografts. We created a Sprague Dawley Rag2 -/-, Il2rg -/- rat (SRG Rat®) that readily supports engraftment of a variety of human cells, tissues, and tumors. The SRG rat lacks B, T, and NK cells and is more immunodeficient than the Nude rat.

Here we show comparative pathology between the SRG rat and its parental strain, the CRL Sprague Dawley (CD® Rat). We also present data from an efficacy study in tumor bearing SRG rats, demonstrating that serial blood draws and tumor biopsies can be performed in tumor bearing animals to assess drug pharmacokinetics and pharmacodynamics within a single study.

These data demonstrate that the SRG rat is suitable for toxicology studies and has comparable pathology to the CD rat, except for reduced lymphoid tissue and WBCs, which are expected phenotypes. In addition, the SRG rat supports the growth of human cancer tissue for treatment studies. The large size of the SRG rat and comparative pathology to the CD rat support its value as an immunodeficient model for assessing toxicity during efficacy testing for de-risking safety concerns in the presence of the human target tissue.

The SRG Rat®

- Sprague Dawley *Rag2* null, *Il2rgamma* null ***SRG rat®.***
- Lacks mature B, T, and circulating NK cells, the most immunodeficient rat available
- High take rates with a variety of human cell lines
- Serial tumor biopsies and blood draws
- Option to perform PK studies within efficacy studies
- Suitable for safety and toxicity testing

Materials and Methods

Ten SRG rats (CRL strain #707; 5/sex) and 10 CD rats (CRL strain #001; 5/sex) at 8-10 weeks of age were necropsied, blood and urine obtained for clinical pathology, and major organs/tissue examined microscopically for comparative pathology. Formalin-fixed tissues from all 20 rats were submitted to Dallas Tissue Research, processed to paraffin blocks, and sectioned at ~4 µm. H&E histoslides were evaluated under light microscopy by an ACVP board-certified veterinary pathologist. Complete blood count (CBC), clinical chemistry, clotting profile, and urinalysis were performed on samples collected on the day of sacrifice by IDEXX Laboratories, with data supplied to the pathologist for interpretation.

VCaP human prostate cancer cells (10 million) were inoculated subcutaneously into SRG rats to form tumor. Fine needle aspirates were taken from tumors weekly for Western blot. Blood was collected weekly for serum PSA measurement by ELISA.

Results: Necropsy and Organ Weights

	Sex	Males		Females	
		CD	SRG‡	CD	SRG‡
Spleen					
	Absolute Weight (g)	0.63	-63.59	0.49	-58.23
	Body Weight Ratio (%)	0.002	-60.13	0.002	-57.03
	Brain Weight Ratio (%)	0.29	-59.52	0.25	-50.04

‡ Mean absolute organ weights and organ weights relative to body and brain weight for CD rats (actual values) and SRG rats (expressed as % decrease from CD rats).

Table 1. Body and Organ Weights. Mean body weights were lower in male (-28%) and female (-48%) SRG rats; however, they were within historical reference range for 8-10 week old CD rats. Thymus was not detected macroscopically in SRG rats. Spleen weights (absolute and relative) were lower in SRG rats compared to CD rats ($p \leq 0.05$). No other relative organ weight or gross pathologic differences were observed between SRG and CD rats for the remaining organs/tissues evaluated (i.e., eyes, cecum, colon, duodenum, heart, ileum, jejunum, kidneys, liver, lung, stomach, and trachea). See below for other lymphoid tissue.

Results: Clinical Pathology

	Sex	Males		Females	
		CD	SRG	CD	SRG
WBC count					
	Absolute Count/% Change	11,120	-83.5%	10,580	-87.2%
Lymphocytes					
	Absolute Count/% Change	9091	-91.8%	7993	-92%
Monocytes					
	Absolute Count/% Change	363.2	-56.8%	375.2	-73.1%
Eosinophils					
	Absolute Count/% Change	89.2	-82.1%	102	-88.2%
Basophils					
	Absolute Count/% Change	18.6	-79.6%	8.6	-32.6%
Neutrophils					
	Absolute Count/% Change	1558	-41.0	2102	-71.4
Mean Corpuscular Volume					
	Fl/% Change	68.6	-21.6%	67	-15.8%

Table 2. Clinical pathology results for CD rats (absolute counts per µL for all except for MCV expressed in fL) and SRG rats (expressed as % decrease from CD rats). Reduced lymphocytes were the most prominent change in the SRG rat. Lymphocyte counts were decreased by 92% in SRG rats relative to CD rats ($p \leq 0.05$). In addition, total WBCs, monocytes, eosinophils, and neutrophils were also significantly reduced in the SRG rat compared to CD rats ($p \leq 0.05$). The difference in basophils between the two strains was not significant. Red blood cell size was slightly smaller in the SRG rat versus CD rats, but within normal range found in CD rats. Other RBC parameters were similar to CD rats. All parameters for clinical chemistry, urinalysis, and coagulation were similar and within normal ranges for SRG rats compared to CD rats.

Results: Histopathology

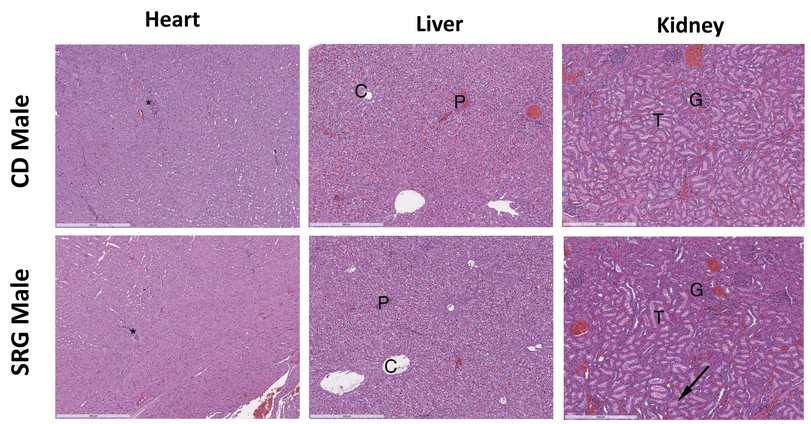

Figure 1. Histopathology of select organs in the SRG rat and CD rat. *H&E Stain.* **Heart, Liver:** Histopathology from CD and SRG rats was within normal limits (black *: blood vessel, C: central vein; P: portal tract). **Kidney:** Minimal tubular cast formation (black arrow) was the only finding in SRG rats and is a common background finding in CD rats. (G: glomerulus, T: tubule). Data not shown: **Spleen:** Lower spleen weights corresponded to grossly smaller spleens in SRG rats. This reduction is size was due to reduced lymphocytes within all regions of the white pulp; the PALS, follicle, and marginal zone seen in the CD rat were absent in SRG rats. The red pulp was not affected. **Bone marrow**: Minimal to mild reduction in cellularity of the hematopoietic tissue was seen in the bone marrow of SRG rats, with a concomitant increase in adipose.

Results: Xenograft Utility

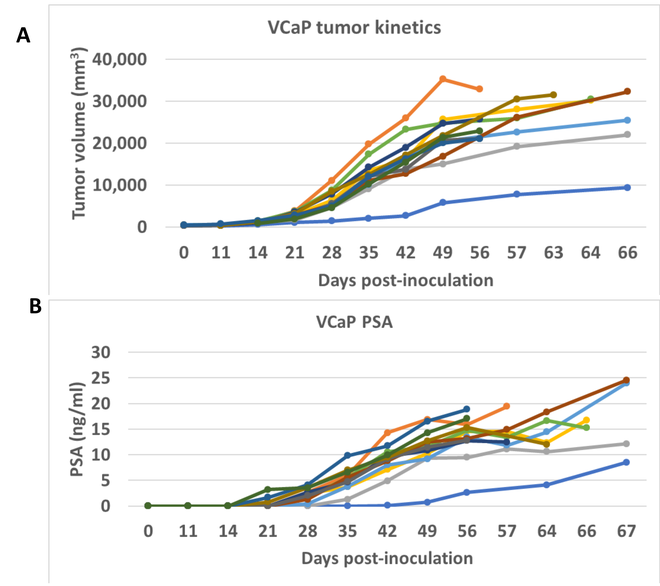

Figure 2. VCaP tumor growth kinetics and serum and tumor PSA in the SRG rat. Ten million VCaP cancer cells were transplanted subcutaneously in the SRG rat. Take rate was 100% in SRG. A) Tumor volume over time. B) Serum PSA assessed weekly throughout the study was highly correlated to tumor volume. C) Western blot for PSA and Androgen Receptor (AR) in tumor biopsies.

Conclusions

Compared with the parental strain (CD rats), age-matched SRG rats displayed lower body weight, but were within the historical reference range for CD rats and had lower spleen weight. Thymus was not identified macroscopically, and mandibular lymph nodes were not identified microscopically in the SRG rat. Lymphoid tissue in spleen, lung, and intestine was not observed in the SRG rat. Loss of lymphoid cells/tissues was the main difference between the SRG rat and CD rat strains. There were no other gross abnormalities macroscopically or microscopically between the SRG rat and the CD rat. The SRG rat supports the growth of human tumor xenografts and is amenable to serial blood collections and tumor biopsies for biomarker analysis.

References

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