Novel immunodeficient rat models offers a unique platform for drug efficacy studies in human tumor xenografts

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Bisoye Towobola[1], Fallon K. Noto[1], Kamesh Ravi[1], Angela Arey[1], Christopher McClain[1], Goutham Narla[2], Christopher Chengelis[1], Jack Crawford[1], and Tseten Yeshi[1]

1 Hera Biolabs, Inc. Lexington, KY 2 Case Comprehensive Cancer Center, Cleveland, OH

Abstract

Immunodeficient mouse models seek to recapitulate the clinical features of advanced human cancers. However, drug efficacy testing and downstream analysis such as pharmacokinetic (PK) /pharmacodynamic (PD) based studies are limited because of variability in tumor growth and kinetics, limited tumor growth potential, requiring the enrollment of larger numbers of mice in each treatment group to achieve required cohort sizes. Considering the distinctive advantages of rats in terms of their larger size, and ease of sampling at different time points for drug efficacy and PK/PD testing, we have developed unique immunodeficient rat models: the *Rag2* knockout SDR™ rat (absence of mature B and T cells) and the *Rag2/Il2rg* double knockout SRG™ rat (absence of mature B and T cells, and lower NK cells) on a Sprague- Dawley background. Our group has previously demonstrated higher engraftment, better tumor kinetics, and increased tumor volume of xenografts in these rat models compared to available immunodeficient mouse strains for various cancer xenograft models. The purpose of the present study is to demonstrate the feasibility of drug efficacy studies using targeted molecular agents directed against cancer- associated drivers in our newly developed SDR and SRG rat models.

We tested the efficacy of a combination treatment of an AKT inhibitor (MK2206) and MEK inhibitor (AZD6244), in a SDR rat xenograft model of human KRAS-mutant non-small cell lung cancer. 1x 10^6 H358 cells were transplanted subcutaneously on the left hind flank in SDR rats. When tumor size reached 100-150 mm^3, the rats were randomized to either control or treatment group. The treatment group received a combination of AZD6244 with MK2206, and the vehicle only control received n, n-dimethylacetamide + Solutol®/Kolliphor® HS 15, twice daily for 30 days by oral gavage. The kinase-inhibitor inhibited the growth of lung tumor xenograft in the SDR rats as shown by decreased mean tumor volume and fold changes in tumor volume compared to the control group. The combination treatment dose was just as effective at half the dose reported in previous mouse studies, demonstrating that lower dosing is sufficient for efficacy testing in rats. Importantly, no mortality, behavioral abnormalities, or changes in body weight were observed during the study, indicating a favorable tolerability profile in this model.

We have also demonstrated that a human prostate cancer cell line VCaP, which has very poor engraftment and growth profiles in existing mouse models, grows well in our SRG rats. The SRG rat is now being used to study the chemotherapeutic efficacy of the benchmark androgen receptor inhibitor Enzalutamide, in this VCaP tumor xenograft model.

Materials and Methods

Generation of SDR™ (Sprague Dawley-Rag2 KO) and SRG™ (Sprague Dawley-Rag2;Il2rg KO) rats: SDR™: the Rag2 locus was targeted using XTN™ technology in spermatogonial stem cells (SSCs). Pooled SSCs were transplanted into DAZL-deficient sterile males and mated with wild-type Sprague Dawley rats. DNA was isolated from offspring and a male with a 27bp deletion was detected. SRG™: the Rag2 and Il2rg loci were targeted using CRISPR via PNI.

Transplantation of human cancer cell lines: The specified number of cells for each cell line were mixed with Geltrex® or Cultrex® 1:1 and transplanted subcutaneously in the hind flank. H358 NSCLC KRAS mutant - 1, 5, or 10 million. VCaP prostate cancer cells – 10e6. Tumors were measured three times weekly and recorded in StudyLog to determine tumor growth kinetics. Animals were euthanized when the tumors reached humane endpoints.

Immune humanization with PBMCs: Human PBMCs were purchased from Stem Express. 50e6 viable cells as determined flow cytometry analysis using propidium iodide were transplanted via the tail vein in SRG rats at 8-10 weeks of age. FACS analysis was performed at 3, 7, 14, 28, 56 and 70 days to assess circulating human CD45+, human CD3+, human CD4+, human CD8+ and human CD20+ cells.

Results

Figure 1: Enhanced survival of NSCLC cell line H358 and tumor kinetics in the SDR™ rat compared to mice

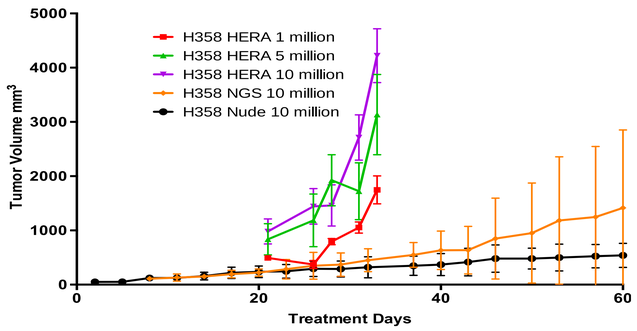

Figure 1. H358 tumor growth in the SDR™ rat compared to the nude (nu/nu) and NSG™ mice. H358 cancer cells were transplanted subcutaneously in the SDR™ rat. Three groups of 6 rats received either 1e6, 5e6, or 10e6 cells in 5mg/ml Geltrex®. Growth rate was directly proportional to the amount of cells transplanted. Take rate was 100%. These data are displayed in conjunction with data from the lab of Dr. Goutham Narla showing tumor growth kinetics of the H358 cell line in Nude and NSG™ mice, both of which were transplanted with 10e6 cells subcutaneously.

Figure 2: Drug efficacy comparison in H358 tumor in SDR™ rats.

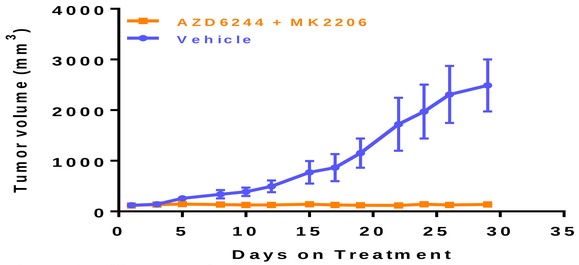

Figure 2. Efficacy study in the SDR™ rat. The antitumor effects of the combination therapy 12mg/kg AZD6244 + 6mg/kg MK2206 (Group 2) was tested in SDR rats. Group 1; DMA: Solutol: Water (1:1:8) was the vehicle control group. N=8 for all groups. All rats received 1e6 cells in the flank. Tumor engraftment rate was 100%. AZD6244 + MK2206 had an apparent anti-tumor effect and was very well tolerated.

Figure 3. VCaP tumor growth in SRG™ rats.

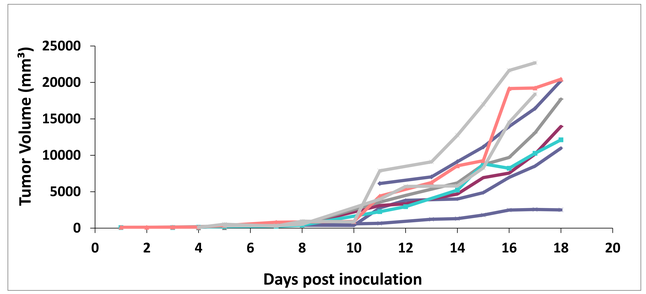

Figure 3. VCaP tumor kinetics in the SRG™ rat. VCaP cancer cells known to have poor engraftment rates and kinetics in immunodeficient mice, thrived in the SRG™ rat. The cells were inoculated in the flank region at a density of 10e6 cells per rat in a 1:1 solution of cell culture media and 5mg/ml Cultrex. Engraftment rate was 75%**.** Each line represents growth in a single animal.

Figure 4: Drug efficacy in VCaP in SRG™ rats

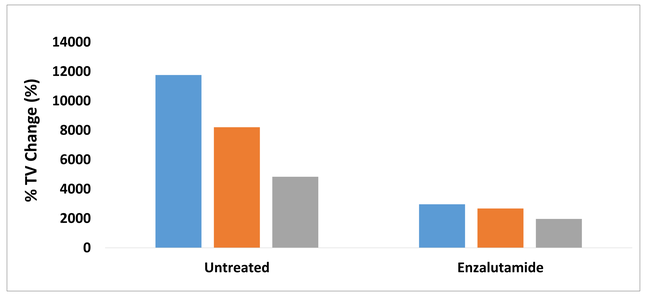

Figure 4. VCaP tumor efficacy. The antitumor effect of Enzalutamide against the VCaP tumor was assessed. Three SRG™ received 30mg/kg of enzalutamide orally once daily. The plot shows reduced percent change in tumor growth rate after 10 days on the drug.

Figure 5: Humanization of the SRG™ rat immune system with PBMCs

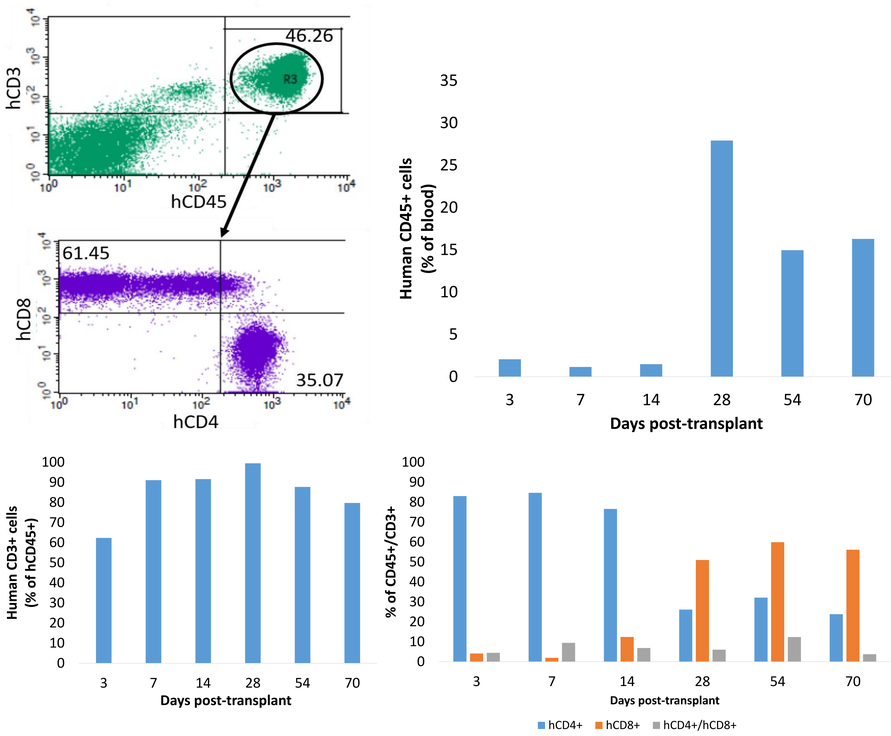

Figure 5: Immune humanization of the SRG™ rat with human PBMCs. Cryopreserved human PBMCs were purchased from Stem Express. Viability was determined by staining the cell suspension with propidium iodide and analyzing on a flow cytometer. 50e6 viable human PBMCs in 500µl PBS were injected into the tail vein of 3 male and 3 female SRG™ rats at 8-10 weeks of age. Peripheral blood was analyzed for the presence of human CD45+, CD3+, CD4+, and CD8+ cells at 3, 7, 14, 28, 54, and 70 days post-injection. By 4 weeks post-transplant, recipients had an average of 29% circulating human CD45+ cells. As of 10 weeks post-transplant, recipients have up to 46% human CD45+ cells and remain healthy. This study is currently ongoing to assess longevity of the human cells and the incidence of graft vs. host disease.

Conclusions

1. Both the SDR™ and the SRG™ rat models support the growth of various human cancer cell lines.
2. The NSCLC KRAS mutant cell line H358 has 100% take rate when transplanted in the SDR™ rat and shows faster and more uniform growth kinetics compared to growth in the Nude and NSG™ mouse.
3. VCaP, a cell line that has poor take rate and growth kinetics in the mouse models, had a very high engraftment and growth kinetics in the SRG™ rat.
4. The SRG™ is permissive to immune humanization with PBMCs, which could provide a model for immuno-oncology efficacy studies.

Acknowledgements

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