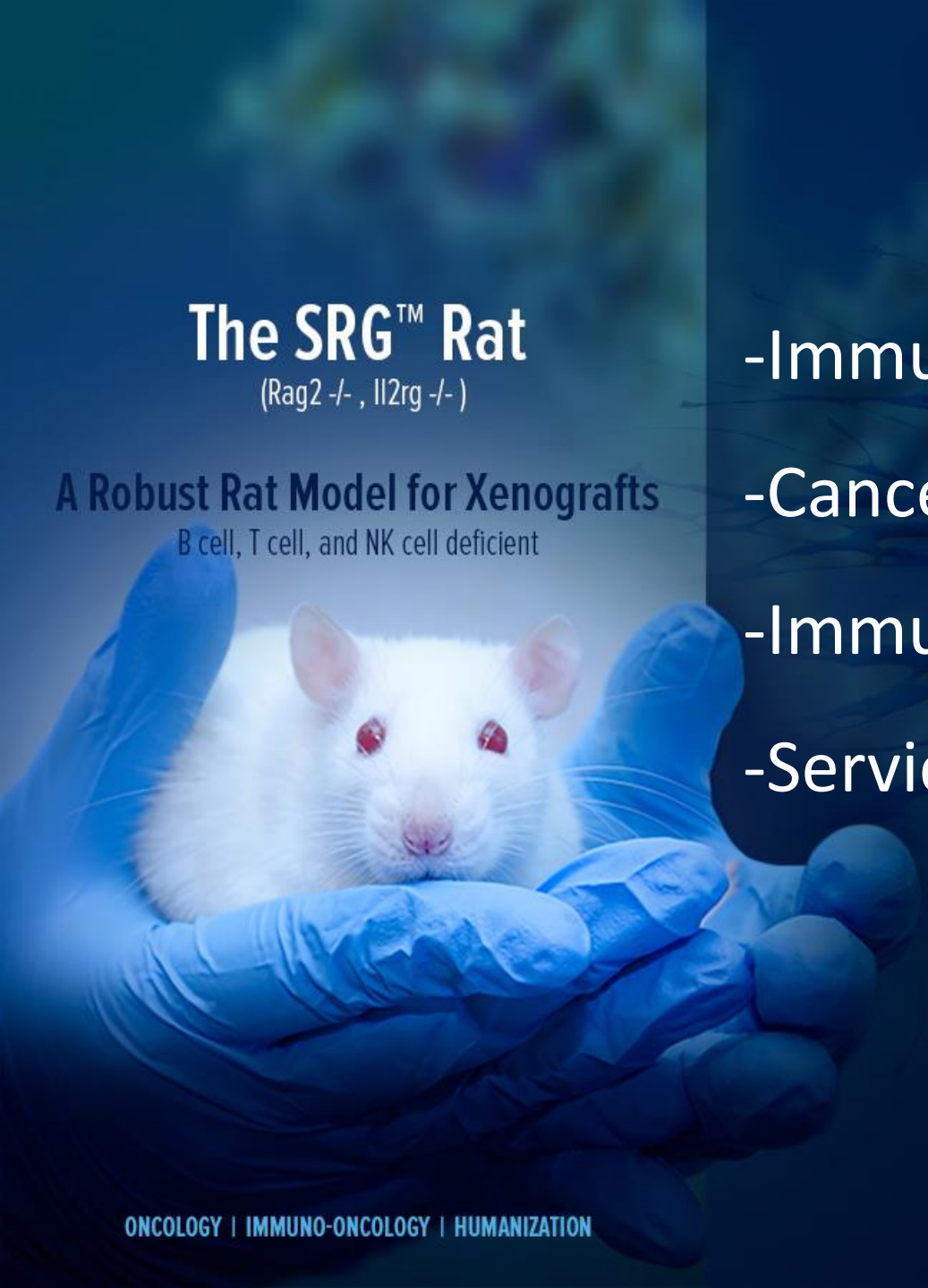
hera BioLabs

The SRG™ Rat

(Rag2 -/- , Il2rg -/-)

A Robust Rat Model for Xenografts

B cell, T cell, and NK cell deficient



ONCOLOGY | IMMUNO-ONCOLOGY | HUMANIZATION

-Immune phenotype

-Cancer xenografts

-Immune system humanization

-Services

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About Hera BioLabs

Precision Toxicology & Efficacy: utilizing precisely gene-edited models such as SCID rats, humanized rodents and engineered cell lines for producing more rapid, consistent and clinically-relevant data

2015	2016	2017	2017/18 plan
Hera spun-out of Transposagen & licenses IP for gene editing technology; development of SCID rats begins; awarded phase II SBIR grant	Completion of a 10,000 ft^2 facility; Scientific team assembled with *in vitro* & *in vivo* efficacy & toxicity capabilities	Introduction of SRG rats and efficacy services; Engineered HepG2 and MDCK cells; *in vivo* toxicity studies and humanized liver mice; custom gene editing, breeding and screening services in mouse and rat	Humanization of the liver & immune system of SRG rats for toxicity and immuno-oncology services

Hera's products & services

The SRG Rat

Cell line & PDX Services

Immuno-oncology in humanized SRG rats & mice

Toxicology services in humanized liver mice

HepG2-CYP Cell Panel

Links for specific product and service information above

Why use SRG rats for xenograft efficacy studies?

Key differentiator

- In mice, many tumor models do not engraft or have very low and inconsistent take rates and growth kinetics, thus conducting a robust efficacy study is not feasible. Hera's SRG Rats have demonstrated better tumor take rates and growth kinetics compared to mouse models, providing more rapid and consistent data.

Additional benefits

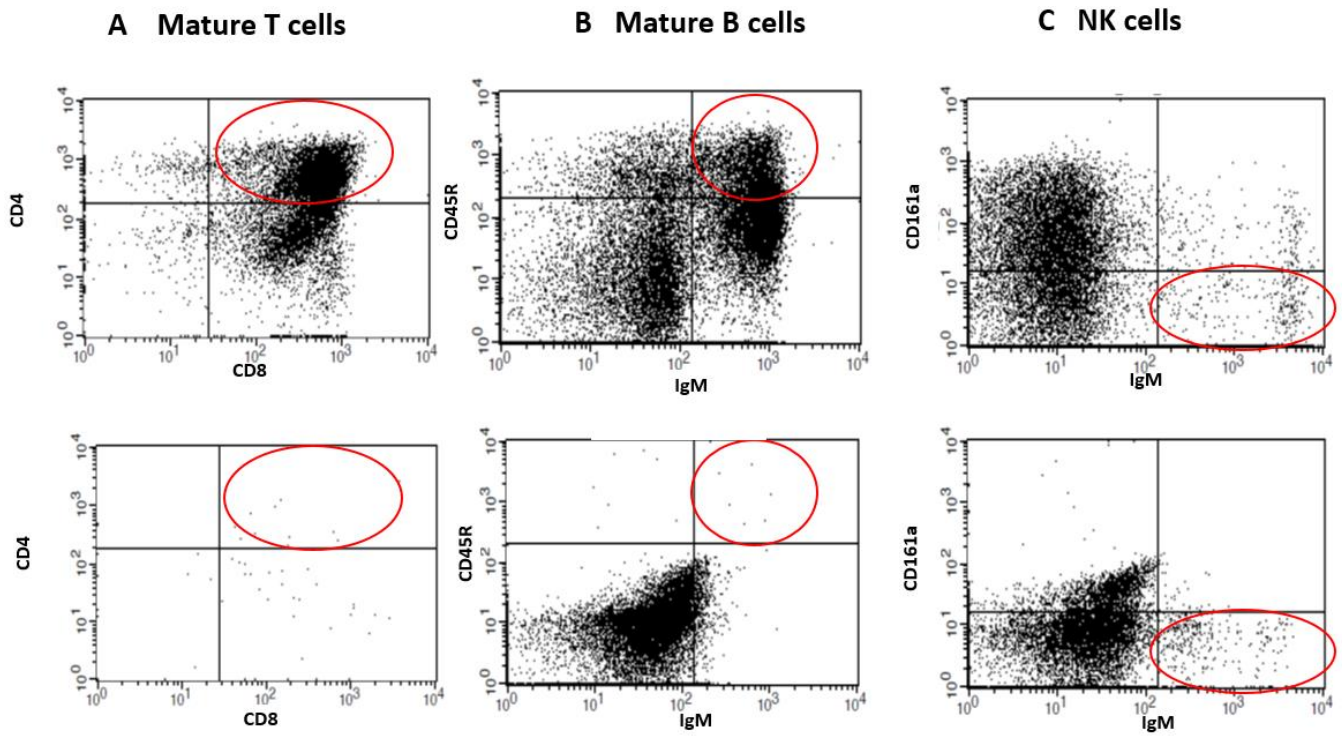
- The rat has a more human-like metabolism and since it is the preferred model for PK/PD and toxicology studies it allows for efficacy to be conducted in the same species (or same animals) as pharmacology/toxicology studies. Traditionally, efficacy studies would take place in mouse models with the corresponding safety studies being conducted in rat; the resulting data comparisons are less than ideal.
- The rat's size allows for large tissue/tumors samples for analysis. The SRG rats can grow much larger xenograft/PDX tumors compared to the mouse and the rats larger size allows for serial collection of samples/blood which enable investigators to have more tissue/blood for analysis or reduce the number of animals needs.
- The generally slower metabolic rate (resulting in lower dosages) and the use of fewer animals mitigates the increased amounts of test article that might be expected when switching from the mouse to the rat.

Hera's SRG rat model

SRG Rats (SD-Rag2-Il2rg KO):

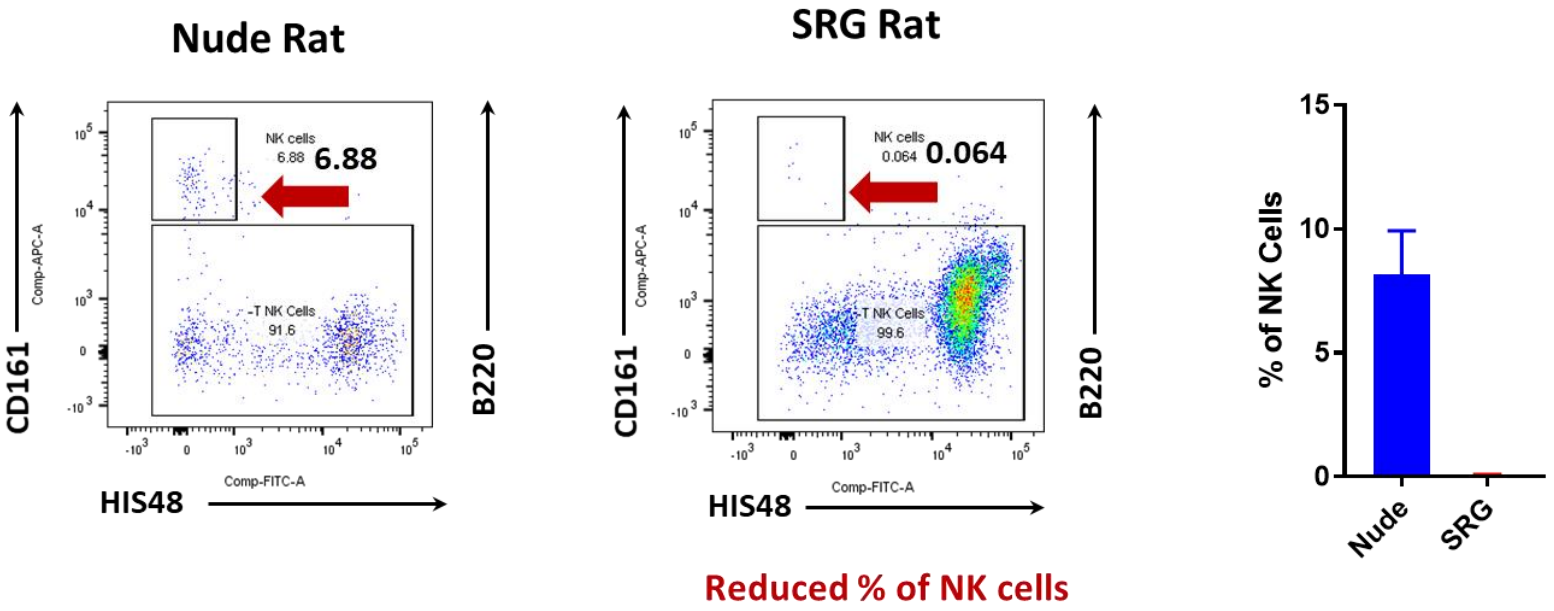
- The most immunodeficient rat model available.
- Knockout of the **R**ag2 (recombination activating gene 2) gene impairs V(D)J recombination and results in a loss of mature B cells and T cells.
- The Il2rg (interleukin 2 receptor **g**amma chain) gene knockout leads to a lack of cytokine signaling, resulting defective lymphoid development.
- The combined mutations result in a loss of mature B, T, and NK cells.
- The background strain is **S**prague **D**awley.

Immunophenotype of SRG rats



Analysis of immune populations in SRG rats. A) CD4+/CD8+ mature T cells are absent from SRG rat thymocytes (bottom panel), compared to a wild-type control (top panel). The lack of thymus tissue in the SRG rat results in a low recovery of thymocytes. B) The SRG spleen contains no mature B cells as demonstrated by lack of CD45R (B220)+/IgM+ cells (bottom panel), compared to WT spleen (top panel). C) The Il2rg knockout in the SRG rat results in a reduced NK cell population (bottom panel) compared to the SDR rat, which only has a Rag2 knockout (compare to figure 1, panel C). NK cells in the SRG rat are similar to or less than the amount of NK cells in the WT rat (top panel).

NK Cells in SRG Rats vs. Nude Rats

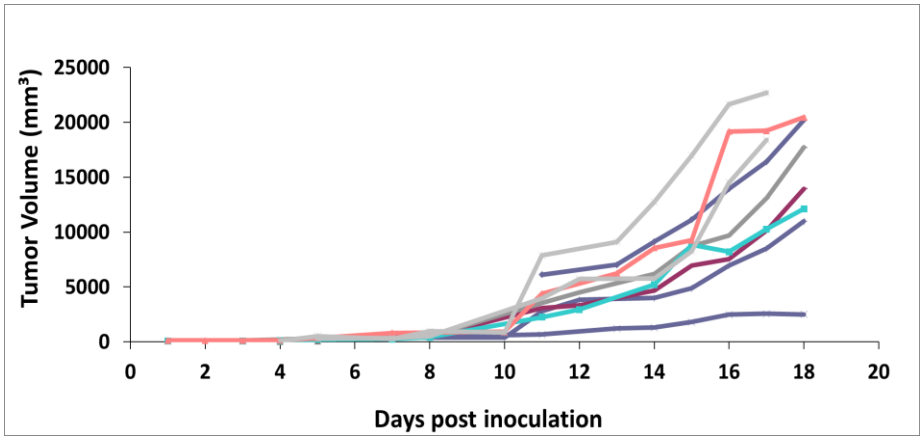


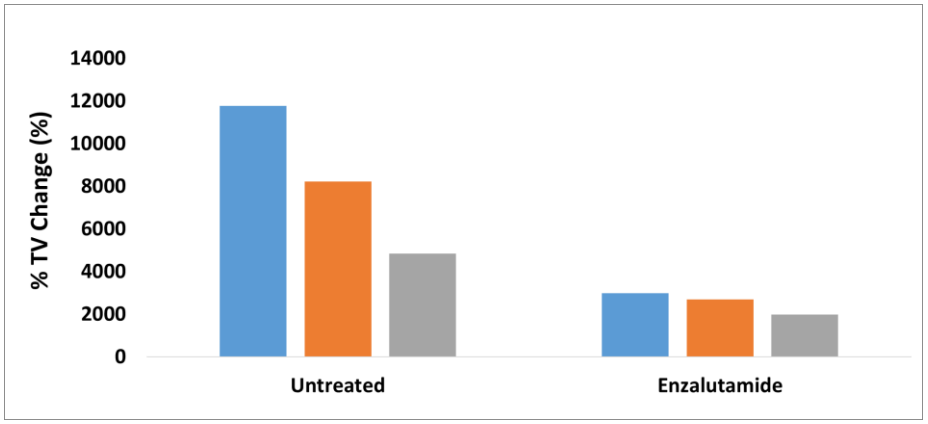
Reduced % of NK cells

Comparison between Nude rats and the SRG rat show a severally reduced number of natural killer (NK) cells.

VCaP Prostate Xenograft in SRG rats - Pilot Study

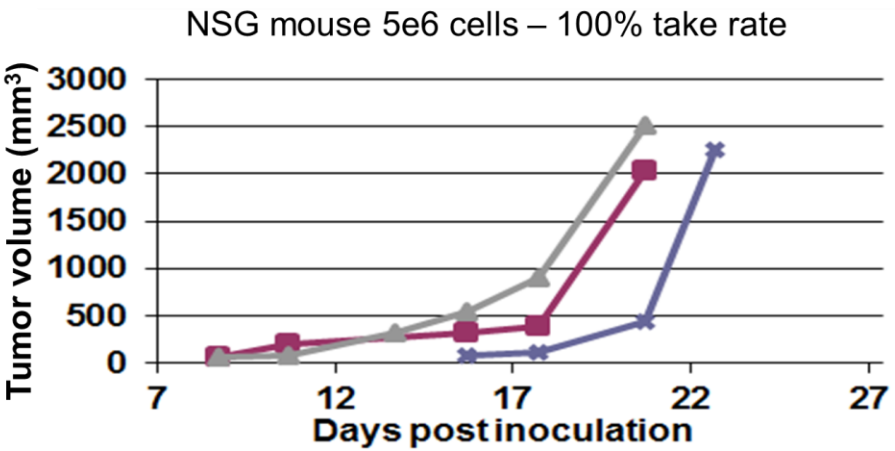
Individual tumor volume of VCaP in SRG rats

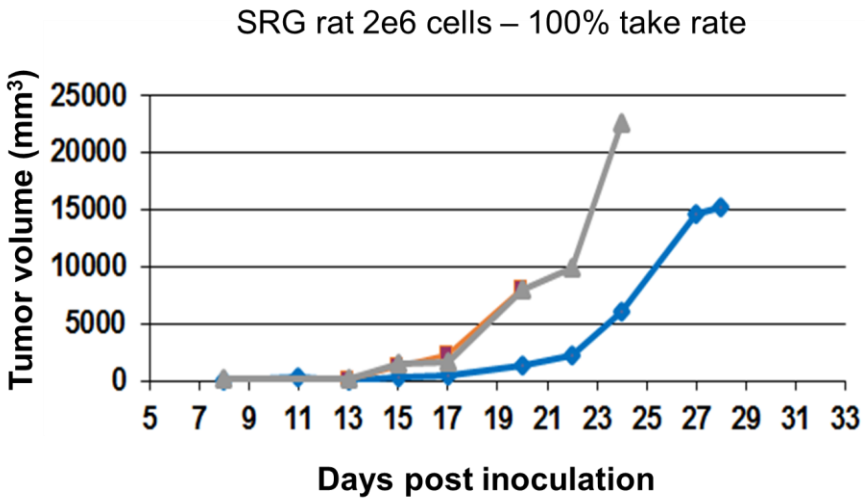




- VCaP exhibits many clinical characteristics including expression of PSA, PAP, and AR.
- Very difficult cell line to grow in mice.
- 75% tumor uptake & favorable tumor kinetics
- Tumor volumes 10x greater than reported in mice.
- Enzalutamide pilot shows efficacy

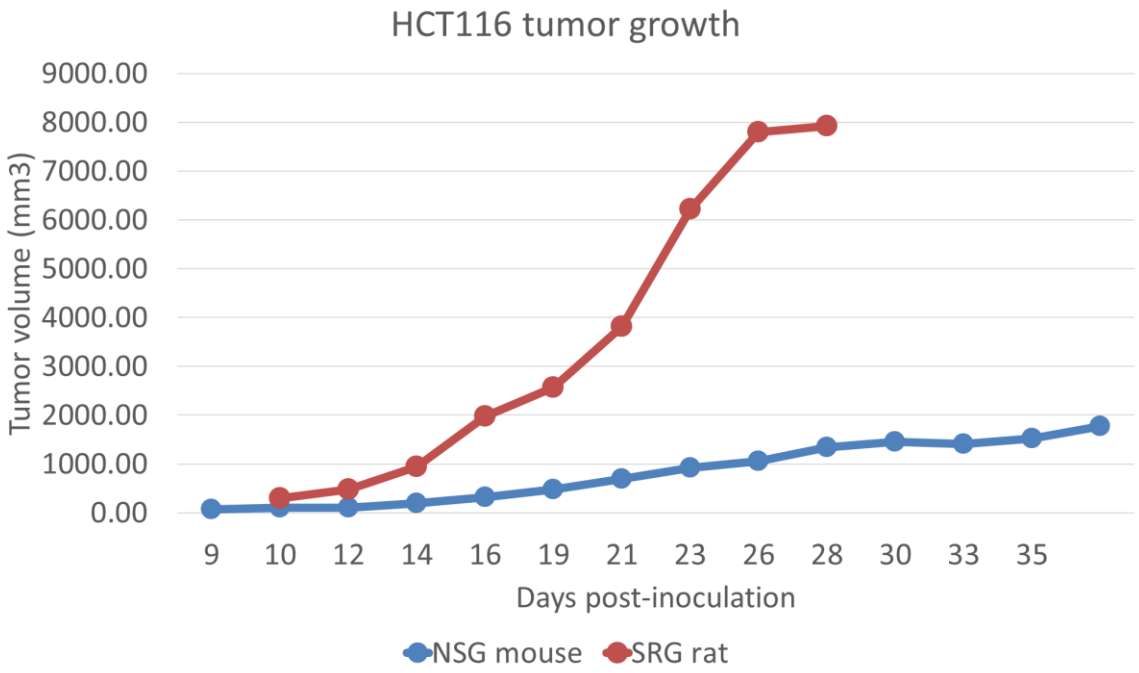
Get to efficacy studies & data faster





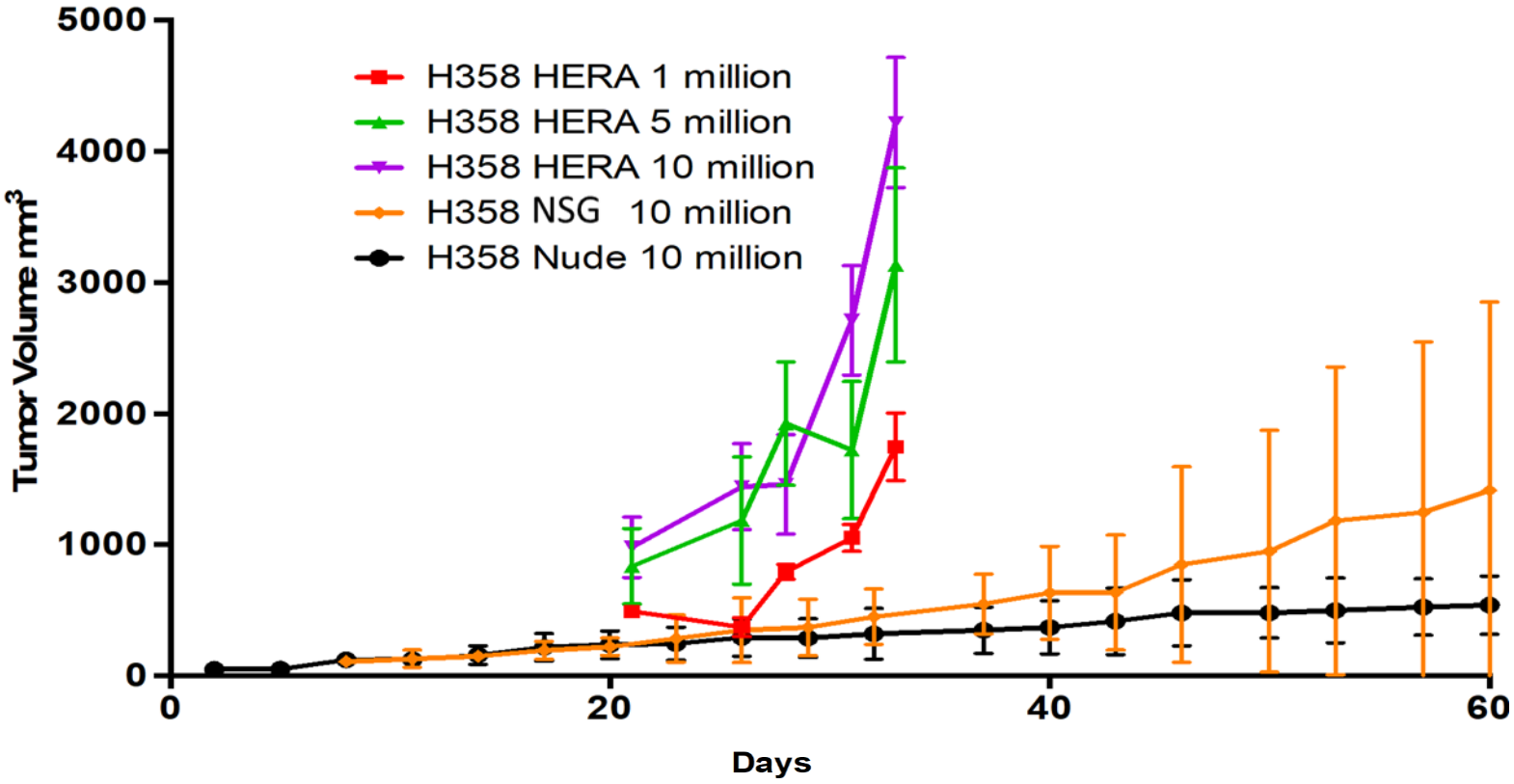
- When a cell line derived from an ovarian PDX model was transplanted SQ the tumor was ten-fold bigger in the SRG rat compared with the NSG mouse, in the same amount of time. The SRG rat thus provides the possibility of getting adequate sample size from PDX tissue for an efficacy study faster than mouse.

Larger tumors - HCT116 Colon Cancer



HCT116 tumor growth in the SRG rat compared to the NSG™ mouse. HCT116 cancer cells were transplanted subcutaneously in the SRG rat and NSG mouse. Six SRG rats and 5 NSG mice each received 2.5e6 cells in 5mg/ml Cultrex® BME 3. Take rate was 100% in both species. Tumor growth rate was significantly larger in the rat compared with the mouse.

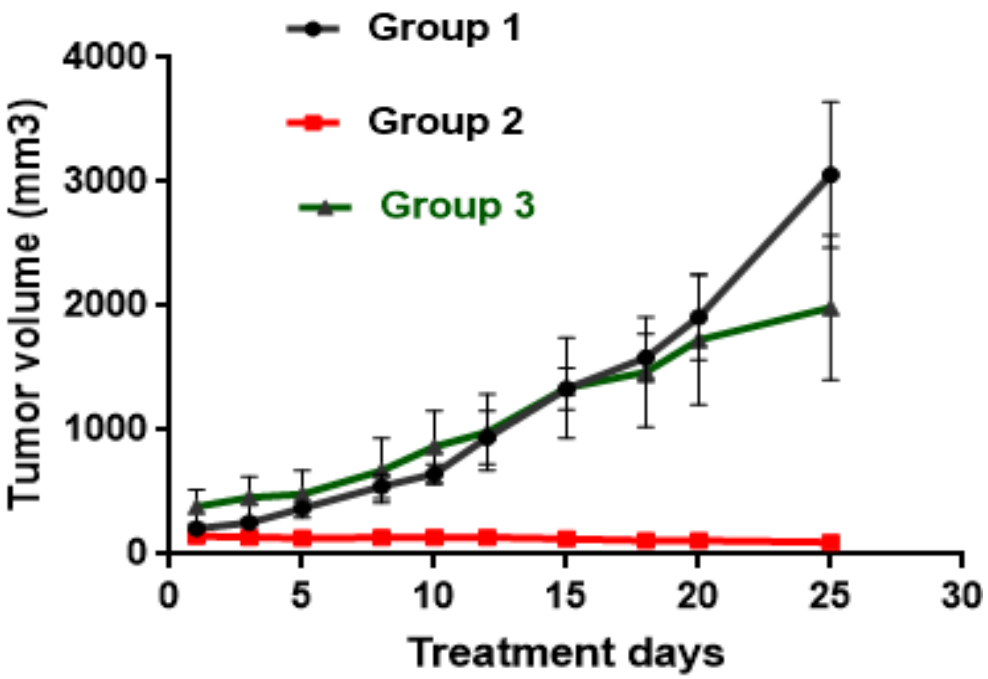
H358 Non-Small Cell Lung Cancer (NSCLC) – KRAS mutant



- 100% engraftment rate and better overall tumor growth kinetics compared to NSG and Nude Mice (~20%) in SDR (Rag2 -/-) rat model. 1, 5, and 10 million cells implanted SQ into the Rat and 10 million cells implanted into the Nude and NSG mice.

H358 NSCLC efficacy study

Mean tumor volume of the treatment groups



- 21 SDR (Rag2 -/-) rats, 1 million cells each
- Treatment groups:

Group 1 : Vehicle control: DMA: Solutol: Water (1:1:8) (n = 6)

Group 2 : 12mg/kg AZD6244 (MEK inhibitor) + 3mg/kg MK2206 (Akt inhibitor) (n = 6)

Group 3: 5mg/kg Test article (n = 6)

- 90% of recipient rats developed tumors

Xenograft/PDX Efficacy Study Services

- Pilot tumor growth kinetics studies
- Efficacy studies
- SRG rats are available off-the-shelf for studies at the clients facility.
- SRG rats pre-implanted with tumor models
- Studies conducted in available mouse models (NSG or SCID) or side-by-side species with Hera SRG rats for comparison studies.

Basic Study Outline	
Model Species:	SRG rats (SD-Rag2-Il2rg KO) NOG/NSG/NCG mice etc.
Length:	28 days
Study Design:	3 test article treated groups, low dose, mid dose and high dose, and 2 controls. 5 males and 5 females for each group.
Dosing Method:	Oral gavage, daily
Tumor Implantation:	Subcutaneous (subQ) or orthotopic
Observations:	Tumor size, bodyweight, food consumption, and clinical observations.
Reporting:	Raw Data, Draft, and Final Report

Inquire for available models

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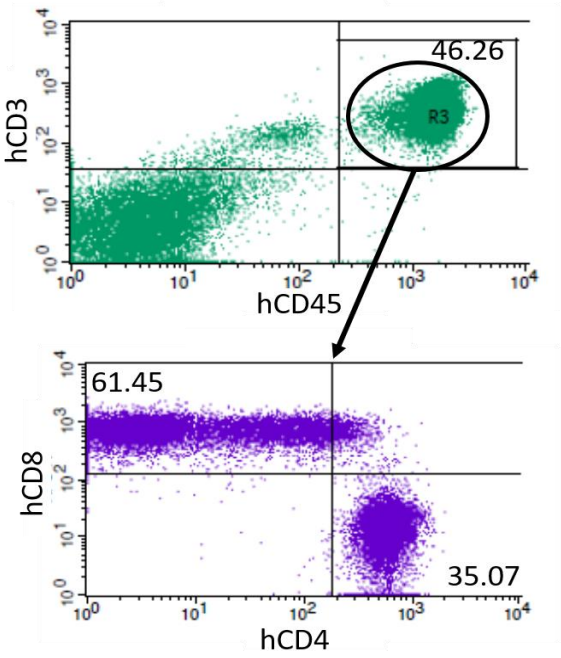
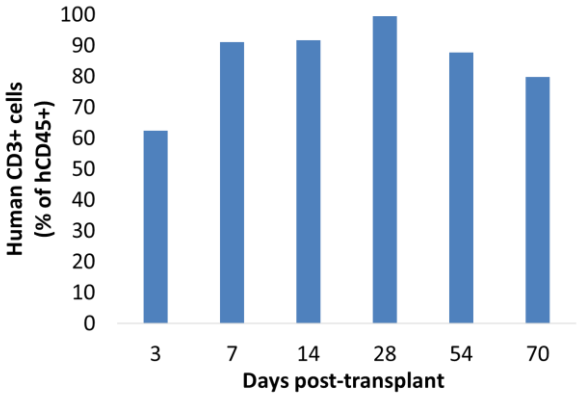
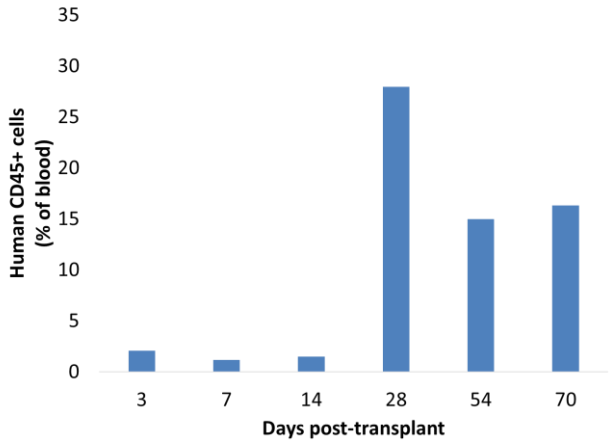
ONCOLOGY | IMMUNO-ONCOLOGY | HUMANIZATION

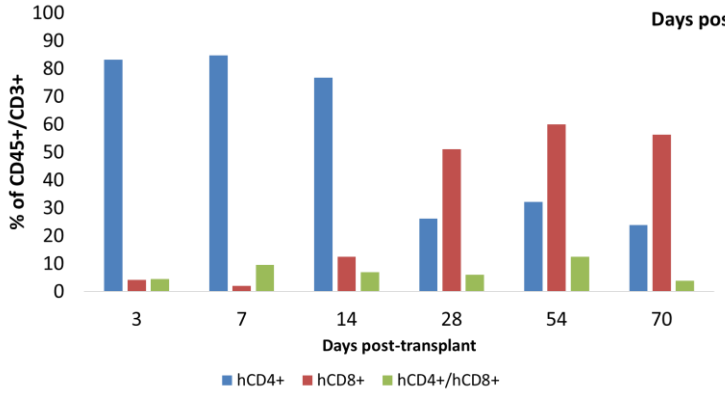
Humanized Immune System SRG rat & mice for immuno-oncology

huPBMC-SRG rat™: Humanization of the SRG rat immune system with PBMCs



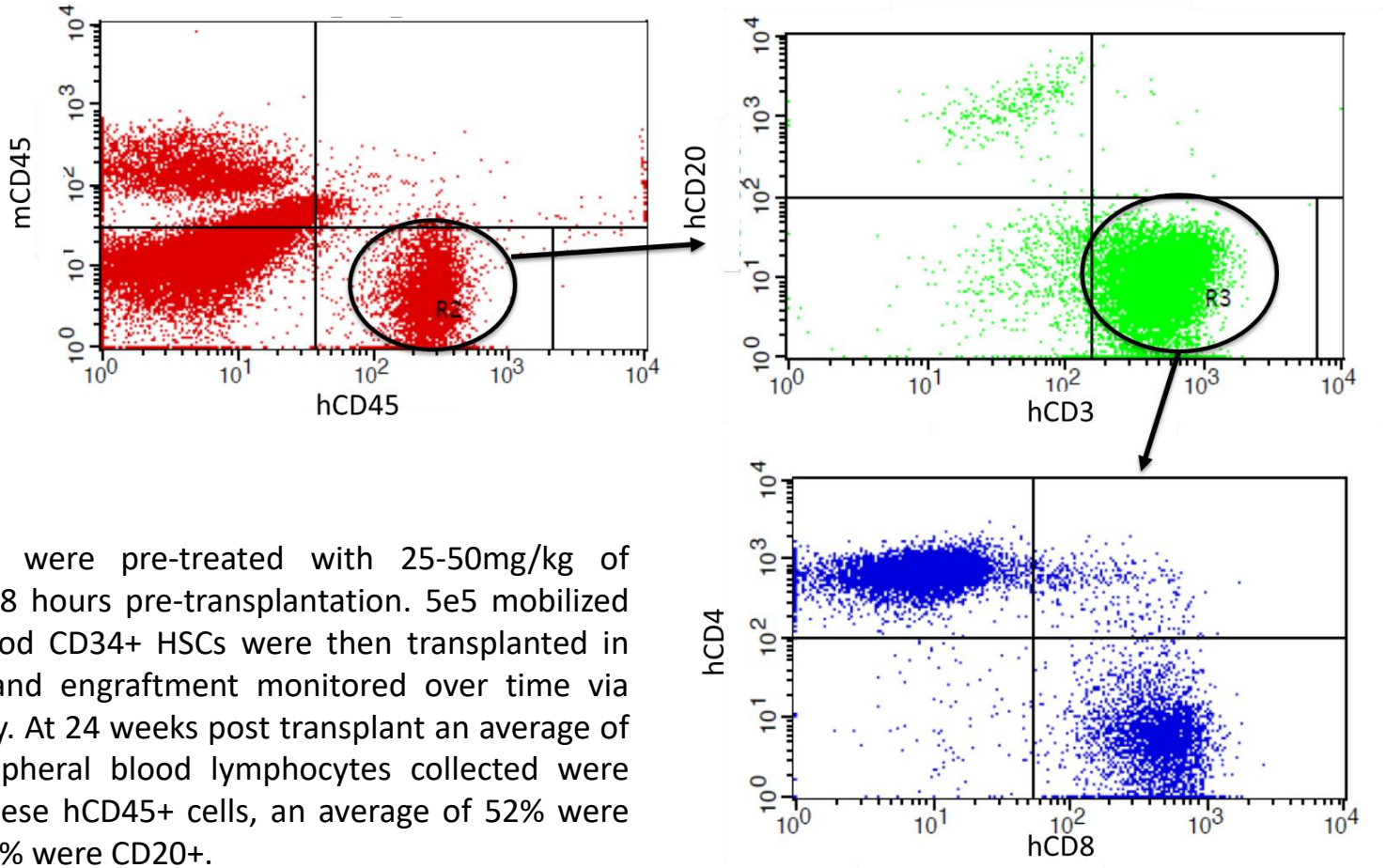






Immune humanization of the SRG rat with human PBMCs. 50e6 viable human PBMCs in 500µl PBS were injected into the tail vein of 3 male and 3 female SRG rats at 8-10 weeks of age. Peripheral blood was analyzed for the presence of human CD45+, CD3+, CD4+, and CD8+ cells at 3, 7, 14, 28, 54, and 70 days post-injection. 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.

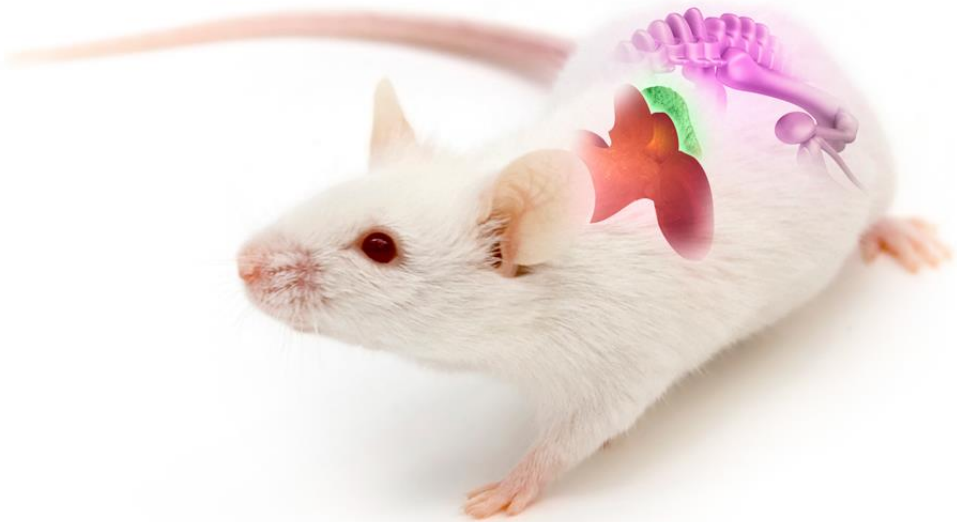
HSC Humanization of NOG mice



5 NOG mice were pre-treated with 25-50mg/kg of busulfan, 24-48 hours pre-transplantation. 5e5 mobilized peripheral blood CD34+ HSCs were then transplanted in each mouse and engraftment monitored over time via flow cytometry. At 24 weeks post transplant an average of 29.4% of peripheral blood lymphocytes collected were hCD45+. Of these hCD45+ cells, an average of 52% were CD3+ and 24.4% were CD20+.

Immuno-oncology services

- Efficacy studies in huPBMC-SRG rat
- Pre-humanized huPBMC SRG rats off-the-shelf
- Efficacy studies in humanized mice (NOG & NSG)



Hera BioLab's Leadership

Jack Crawford, M.S.
VP, BD
Formerly directed the Sales, Marketing, and Business Development Divisions at Transposagen. Experience in product development, licensing, technology and patent evaluation, and fundraising.

Tseten Yeshi, Ph.D.
VP, R & D
Former Director of R&D at Transposagen. An expert in genome editing with well-developed scientific program management skills and experience.

Chris Chengelis, Ph.D., DABT
Senior Scientific Advisor
Former CSO at WIL Research. 35 years+ experience in the preclinical toxicology industry, facility design, study design and execution

Fallon Noto, Ph.D.
Senior Scientist
10+ years working with mice and rats, expertise in rodent humanization, cell and tissue transplantation, microsurgery, and ethical animal care.

Chris Brenzel
Business Development Manager
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Goutham Narla, M.D., Ph.D.
SAB Member & Consultant
The Pardee Gerstacker Professor of Cancer Research and a Medical geneticist at Case Western Reserve University. CSO and Scientific Founder of Dual Therapeutics, Inc. Expertise in cancer genetics and xenograft and transgenic models of cancer with over 58 publications in the field.

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