Immunodeficient rats for cancer xenografts and humanization of the liver and immune system

The use of immunodeficient mice has transformed pre-clinical approaches to screen for efficacy and toxicity. However, the rat offers a number of advantages over the mouse as a research model; it is a larger animal that is metabolically closer to humans making it the preferred model for physiology and toxicology. In xenograft studies, rats can grow larger tumors and produce larger samples for analysis. More precise surgical manipulations and better in-vivo imaging of metastatic tumors are possible. 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 is appropriate for multiple dosing, particularly by routes other than oral, and for collection of multiple blood samples, which enable investigators to assess efficacy and systemic toxicity in a single animal over longer time periods.

The Nude *rnu* line is the most widely used partially immunocompromised rat model. While the Nude *rnu* is deficient in T-cells, it has normal B and NK cells. Partial immunodeficiency of the Nude rat results in a limited number of low efficiency xenografts and makes near-complete "humanization" of specific organs and tissues impossible. In order to overcome the limitations of current immunodeficient rats, two models have been created by Hera. A *Rag2* knockout and a *Rag2/Il2rg* double knockout. The Rag2 KO lacks mature B and T-cells, the double KO further lacks NK cells (figure 1).

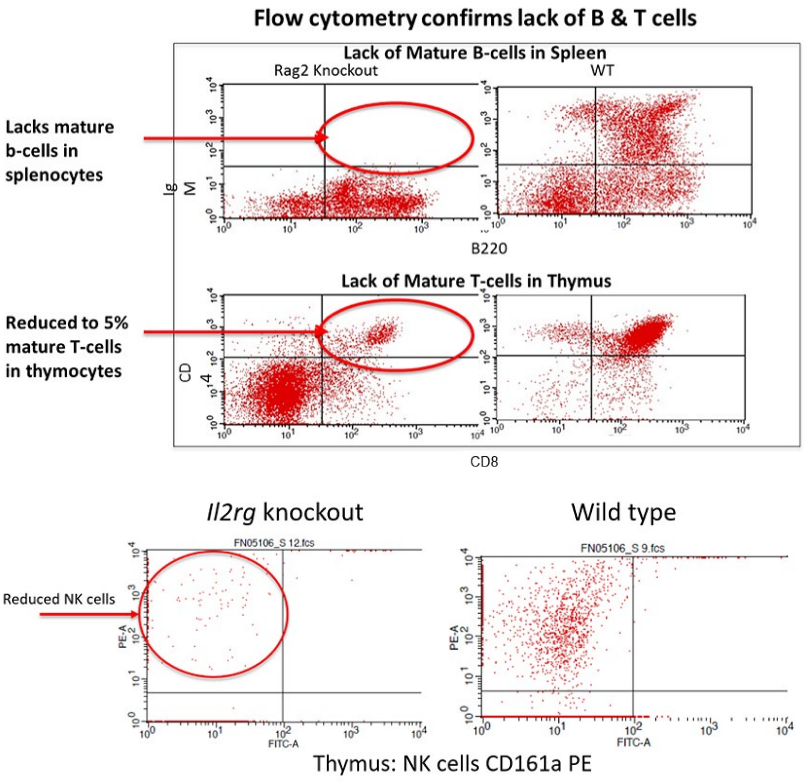

Figure 1: Rag2 knockout animals lack mature B cells and T cells, Il2rg knockouts have depleted NK cells: Splenocytes and thymocytes were collected from age matched homozygous Rag2 knockouts and wildtype animals and were analyzed by flow cytometry. Interestingly, mutant animals were essentially athymic: only residual tissue remained weighing approximately 10% of the a wildtype organ could be identified and collected. Splenocytes were analyzed for the markers B220 and IgM (B cell receptor) thymocytes were analyzed for CD4 and CD8. In Il2rg knockout rats thymocytes were analyzed by flow cytometry for marker CD161a indicating depleted NK cells.

The Rag2 KO demonstrates efficient engraftment of cancer cell lines

The ability to collect multiple larger blood samples in comparison to the mouse makes the immunocompromised rat ideal for blood cancer xenografts. The Reh ATCC® CRL-8286™ acute lymphocytic leukemia (non-T; non-B) cell line was implanted into 3 *Rag2* knockout rats via tail vein injection. Although analysis of splenocytes revealed very low engraftment efficiency, 2 of the 3 rats developed tumors in the spinal cord, lymph nodes and kidney. Immunohistochemistry confirmed that the tumors were indeed human. (figure 2, work done in collaboration with Noble Life Sciences, Woodbine, MD)

The U87MG human glioblastoma cell line was implanted subcutaneously into 3 female and 3 male *Rag2* knockout rats and 3 control Rag2 heterozygous and wild type rats. *Rag2* knockout rats had visible tumors as soon as 10 days post implantation and by day 27, 6/6 *Rag2* knockouts had visible and palpable tumors. At 34 days post-injection, the largest tumor measured 32.1mm in diameter None of the control animals grew tumors. (Figure 3 a-c)

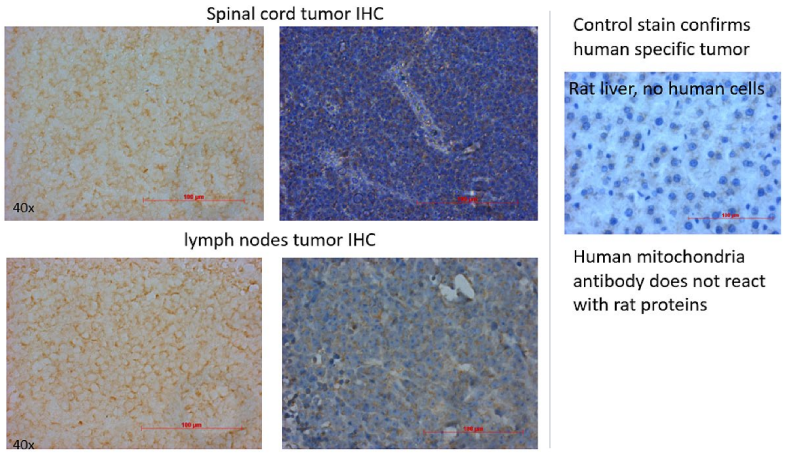

Figure 2: confirmation of human tumors derived from Reh ATCC® CRL-8286™ acute lymphocytic leukemia (non-T; non-B) cell line injection: Immunohistochemistry (IHC) was done on spinal cord and lymph node tumors, brown anti-human mitochondria, peri-nuclear stain identified the tumors as human, hematoxylin (general nuclei stain) shown in blue/purple. Control stain confirms that the human mitochondrial antibody does not react with rat proteins.

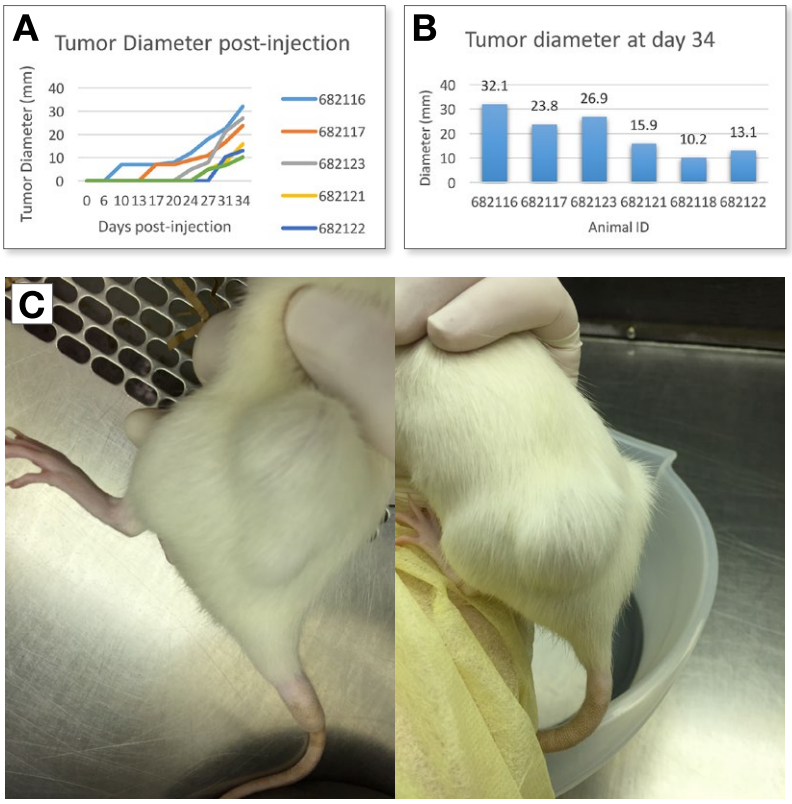

Figure 3: Subcutaneous human glioblastoma-derived tumors in *Rag2* knockout animals. A) Tumor diameter was measured twice weekly after injection of U87MG human glioblastoma cells subcutaneously. 6/6 *Rag2* knockout animals have developed measurable tumors by 27 days post-injection. B) Tumor diameter as of day 34 post-injection. C) Images of visible tumors of two different Rag2 knockout animals.

Future directions - Patient Derived Xenograft (PDX) models & humanization of the Rag2/Il2rg double knockout rat

The *Rag2/Il2rg* double knockout rat is a Severe Combined Immunodeficiency (SCID) model with depleted B, T and NK cells making it an ideal recipient for humanization. Work is ongoing to "humanize" the liver and immune system in this SCID rats by ablating endogenous cells or tissues (i.e. hepatocytes) and replacing them with human counterparts. In humanized rodents, organ function becomes more relevant to human studies; examples include how the animal responds to hepatic toxicity and metabolism. Hera is focusing on humanizing the rat liver and is actively seeking collaborators in immune system humanization development studies.

Patient-derived xenograft (PDX) models, based on the transfer of primary tumors directly from the patient into an immunodeficient model, are now being widely used in cancer research and efficacy studies. The rat's larger size may result in faster expansion of PDX lines. Hera is utilizing PDX in the *Rag2/Il2rg* double knockout and is actively seeking collaborators to run studies as a service as well as sources for PDX models.