Trials and Tribulations of Humanizing Mice for Cancer Research

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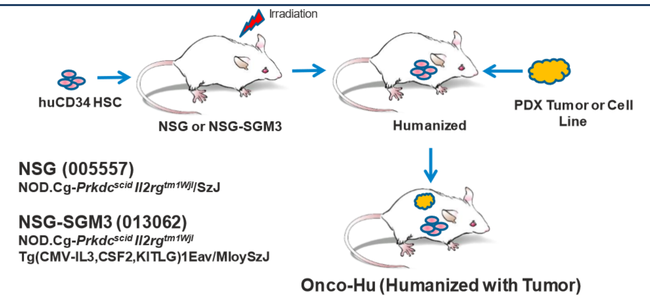
Abstract

Cancers are aggressive, evasive, and ruthless killers, claiming millions of lives every year. Cancers are heterogeneous and there is often no single, clearly defined problem as they harness and manipulate a multitude of fundamental mechanisms at the very essence of life. To investigate these mechanisms and vet potential interventive therapies, humanized mice offer a unique model as a prelude to the use of nanosecond pulse stimulation (NPS), a pulse power technology applying nanosecond duration, high electric field pulses, to ablate human tumors. Immunodeficient mouse strains, NSG and NSG-SGM3, were engrafted with human immune cells and human tumors, which would allow us to study the effects of NPS therapy on the human tumor and the human immune system, albeit not without trials and tribulations. Here we show that mice engrafted with human cord blood CD34+ hematopoietic stem cells (hCD34+ HSC) lack consistency in expansion and chimerism, or variety of immune cell types. Unfortunately, mice that developed the human immune system rejected the human tumors without treatment, while mice that rejected the immune system developed the human tumors. Therefore, we had mice with human immune systems and no tumor to treat, and mice with tumors to treat yet no immune system to study. In non-humanized mice, NPS induced complete tumor death in the patient derived mammary cancer xenograft (PDX) model, but not in the MDA-MB-231 VIM-RFP mammary cancer cell-derived xenograft (CDX) model. The absence of NPS elimination of the CDX is the only known NPS cancer failure and requires further study.

Objectives

- Treat human breast cancer tumors in humanized mice
- Examine the NPS-induced immune response in humanized mice

Methods



Onco-Hu Models: Humanized Mice for Evaluation of Immuno-Oncology Therapeutics. The Jackson Laboratory; 2019.

***Fig.1*. Humanized mouse models.** NSG or NSG-SGM3 mice were engrafted with HCB CD34+ HSC at 4-6-wks old. Mice were treated with sublethal irradiation 4-6-hrs prior to receiving HCB CD34+ stem cells via IP injection. The developing immune system was monitored via regular blood checks. After 10-12-wks, tumors were started in the mammary fat pad of hu-mice. The tumors were treated with NPS (1000, 100ns Blumlein line pulses at 50kV/cm and 3Hz) 16-20-weeks post-HCB CD34+ HSC engraftment.

Results

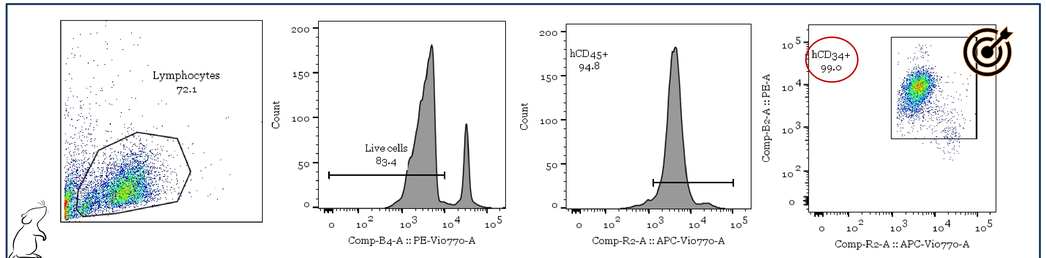


***Fig.2*. Representative analysis of HCB CD34+ stem cells pre-engraftment.** The StemCell HCB CD34+ Stem Cell Isolation Kit was used for positive selection of HCB CD34+ HSC. After cryopreservation, flow cytometry was used to confirm the purity of the isolated cells prior to humanizing mice. Impure samples might include various types of mature immune cells which could inadvertently induce graft-versus-host disease (GvHD) and lead to premature mortality.

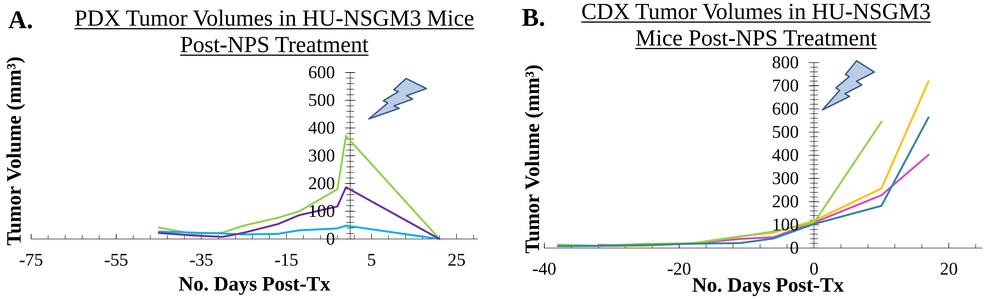
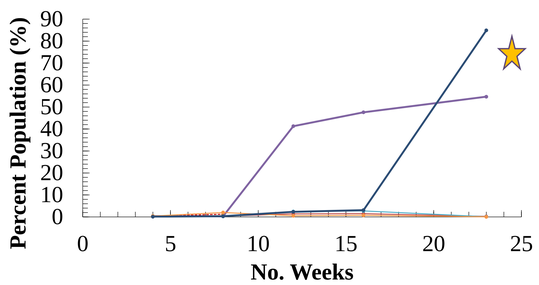


***Fig.3*. Human PDX (A) and CDX (B) tumor volumes in HU-NSGM3 mice post-treatment with NPS.** Tumors were monitored for development and treated with NPS approximately 16-20-weeks post-engraftment of HCB CD34+ HSC. (A) PDX tumors completely regressed after treatment; however, (B) CDX tumors continued to develop after treatment. The progression of the CDX tumor development was the first time NPS has been ineffective at inducing tumor death.

***Fig.4*. Human CD45+ leukocyte expansion over time in HU-NSGM3 mice.** Blood was collected from HU-NSGM3 mice over the anticipated engraftment timeframe. The first indication of humanization is by observing the percentage of human lymphocytes, which are hCD45+ cells, in the blood. Here, you can see that over time, only two of the mice in this group developed human lymphocytes, while the others rejected the engrafted stem cells.



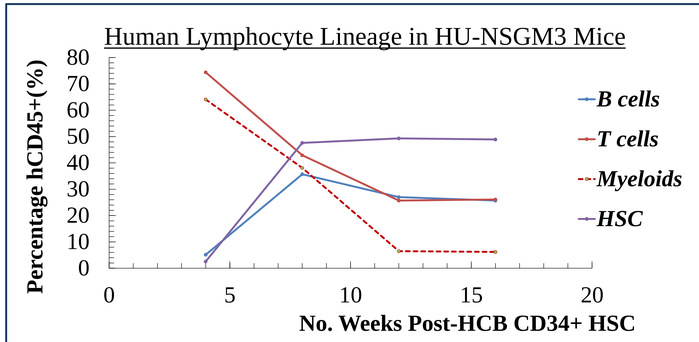
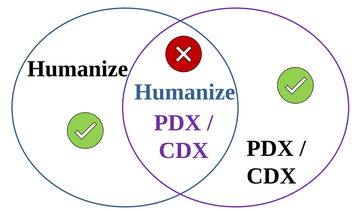


***Fig.5*. Human lymphocyte populations in HU-NSGM3 mice after humanization.** Over time, the engrafted HCB CD34+ HSC expand and differentiate into a variety of human immune cells, including B cells (hCD19+), T cells (hCD3+), myeloid cells (hCD33+), and HSC (hCD38+). However, adequate expansion rarely occurred, and data is shown for a single mouse.

Conclusion

- Inconsistent humanization
  - Humanization rarely occurred
  - GvHD also occurred
- PDX tumors were often rejected
- PDX tumors were cleared with NPS
- CDX tumors did not regress with NPS
- Hu-mice that developed the human immune cells, rejected the PDX
- Hu-mice that rejected the human immune cells, accepted the PDX



This research is brought to you by the Beebe Laboratory and collaborators, with support from the following entities to which we are especially grateful:

Pulse Biosciences, Inc. **The Catalyst – VBHRC**

Special thanks to my lab mates and…

SoBran Vivarium Staff & Veterinarians
Yu Jing,, Ph.D. Piotr Kraj, Ph.D., DVM

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