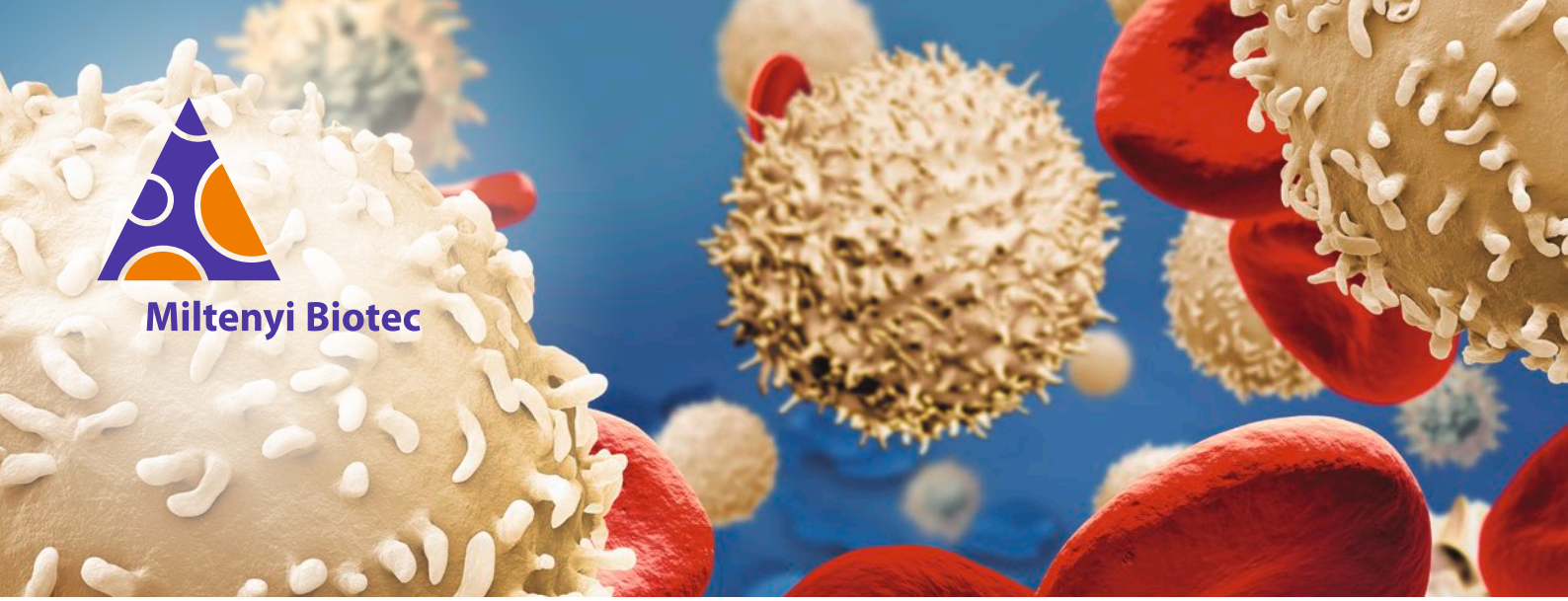

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Generation and preclinical applications of humanized mouse models to study human immunity and cancer immunotherapy

Julie Lang, PhD
Co-Director of the Pre-clinical Human Immune System Mice Core (PHISM)
Assistant Research Professor at the University of Colorado Denver Anschutz Medical Campus, USA

Background

Human immune system (HIS) mice are created by the engraftment of human hematopoietic stem cells (HSCs) and the reconstitution of a functional human immune system in an immunocompromised mouse. Humanized mice constitute a sophisticated preclinical model where the human immune system can be exposed to *in vivo* experimental and therapeutic manipulations providing meaningful drug testing outcomes. HIS mice are increasingly utilized to understand human immune system development, immune responses to human-specific pathogens, even effects of microbiomes on human immunity, and more recent efficacies and mechanisms of combination immunotherapy treatments on human cancers.[1–9]

Here we show an automated experimental setup for human CD34+ HSC enrichment from umbilical cord blood for the successful generation of humanized mouse models. High HSC viability and purity are essential for successful engraftment and human immune system reconstitution in the absence of GVHD. To obtain high quality HSCs, MACS® Technology is the best choice. CD34+ cells can be purified from human cord blood using MACS MicroBeads and the autoMACS® Pro Separator and then transplanted into immunodeficient mice to create powerful mouse models for *in vivo* preclinical evaluation of human immune responses. In addition, human tumors can be implanted in the flanks, orthotopically, or intravenously to also study human immune changes following cancer therapy (fig. 1).

Here we present two examples on the applications of HIS mice:

- Evaluating therapeutic efficacy of immune check point inhibitors on HIS mice engrafted with cell line–derived xenografts (CDX) or patient-derived xenografts (PDX)
- Understanding the effects of antibiotics on gut microbiome diversity in HIS mice

Methods

CD34+ cell enrichment from human umbilical cord blood
Human HSCs were isolated from fresh umbilical cord blood using the CD34 MicroBead Kit, human and the autoMACS Pro Separator, as previously described.[1–4] Cord blood mononuclear cells (MNCs) were isolated via density-gradient centrifugation and CD34+ cells were enriched with the autoMACS Pro Separator using the double-positive selection program PosselD2 for highest purity. Isolated HSCs were analyzed for human CD3 and CD34 expression with flow cytometry. CD34+ cells were expanded in short-term cultures with 10 ng/mL interleukin-6 (IL-6), 20 ng/mL stem cell factor (SCF), and 10 ng/mL Flt3 ligand for 3 to 6 days and cryopreserved until *in vivo* transplantation.

Generation of human immune system (HIS) mice
Human CD34+ cells (2–6 $\times 10^5$ expanded CD34+ cells /mouse) were injected into sublethally irradiated neonate HIS-BALB/c-Rag2^{null}Il2rγ^{null}SIRPα^{NOD} (BRGS) immunodeficient mice. HSC reconstitution in HIS mice was evaluated between weeks 10–16 post-CD34+ cell transplantation by characterizing human and mouse immune cell populations in peripheral blood with flow cytometry.[4] HIS-BRGS mice were randomized into equivalent experimental groups based on human chimerism for downstream applications.

Preclinical validation of immunotherapy in humanized tumor xenograft mouse models

CDXs or PDXs were implanted in 17–20 week old HIS mice for immune-oncology drug testing, as previously described.[4–7] Cells of the triple-negative breast cancer (TNBC) cell line MDA-MB-231 (5×10^6 cells) were injected subcutaneously into both flanks of HIS-BRGS mice. Adrenocorticol carcinoma (ACC) PDX was established in nude mice, explanted and diced tumor tissue was subsequently implanted subcutaneously with a trocar into both flanks of HIS animals. Tumors were allowed to grow until ~150 mm^3 and treatments with anti-PD-1 immune checkpoint inhibitor (Nivolumab) were given weekly.
At least two weeks later, tumors were resected and dissociated using the gentleMACS™ Octo Dissociator with Heaters. Soft and hard tumors were processed using the hTDK1 and the hTDK3 program, respectively. Single-cell suspensions were stained with antibodies against human and mouse CD45 to analyze human immune infiltration within the tumor with flow cytometry.

Effect of gut microbiome on human immune system

The human gut microbiota is considered a major modulator of the immune system during development and in health and disease. To address how the microbiome composition influences immune homeostasis, we manipulated the gut microbiome and interrogated the human immune system in the HIS-BRGS mice. Humanized (HIS) mice and non-humanized (BRGS) immunodeficient mice were left untreated or treated with Septra, a broad spectrum antibiotic. Feces were collected after two weeks of treatment and the microbiome was subjected to 16s RNA sequencing to identify bacterial species. The alpha diversity was determined according to the microbiome lab of Catherine Lozupone at the University of Colorado Denver Anschutz Medical Campus.

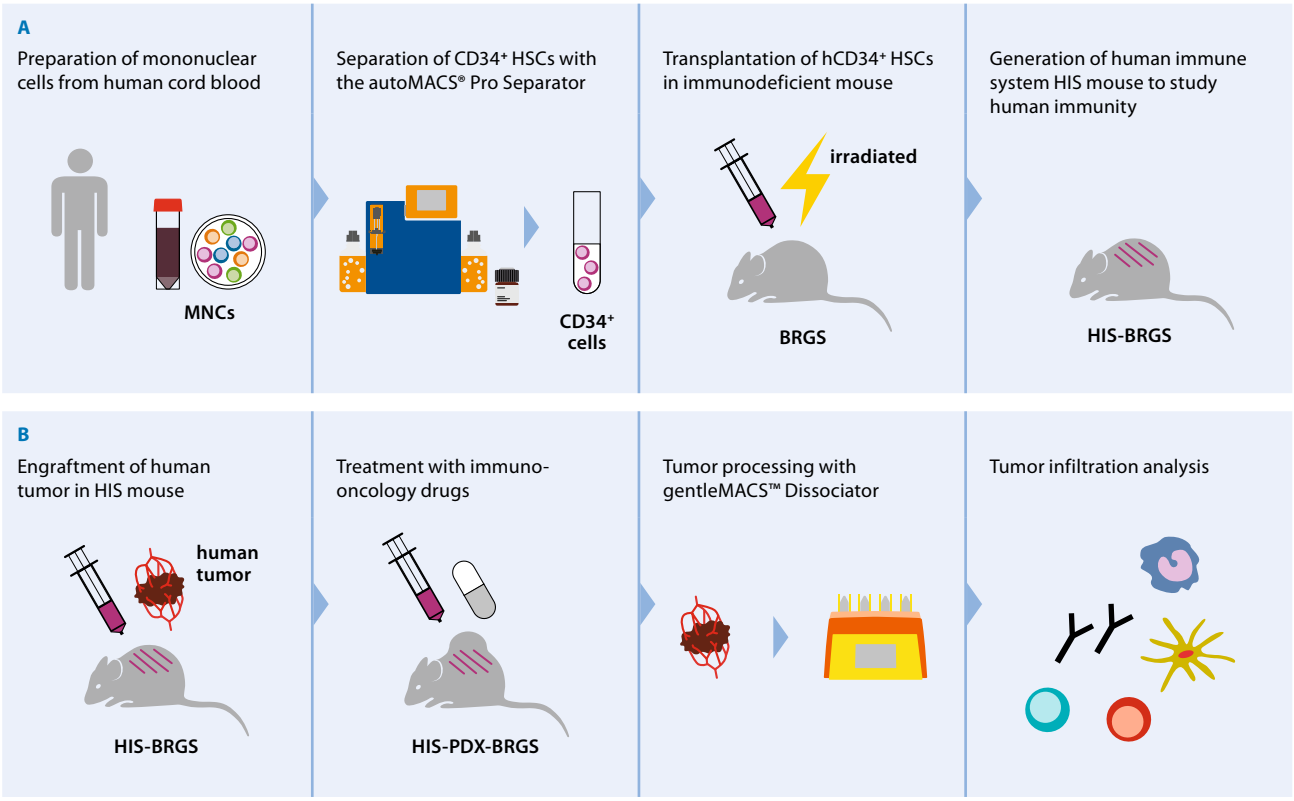

Figure 1: Schematic overview of two workflows for the generation and application of humanized mouse models in cancer immunotherapy. (A) Human HSCs are enriched from umbilical cord blood mononuclear cells with CD34 MicroBeads using the autoMACS Pro Separator. Isolated $CD34^+$ cells are injected into irradiated newborn BRGS immunodeficient mice. Following confirmation of human chimerism, HIS mice are utilized in various applications to study human immunity. (B) HIS mice can be engrafted with human tumors and used to test cancer immunotherapy. Following drug treatments, resected tumors are dissociated on the gentleMACS Dissociator and analyzed for human immune cell infiltration with flow cytometry.

Results

Generation of HIS mice from human cord blood-derived CD34⁺ cells

Human HSCs were isolated from umbilical cord blood with positive selection using CD34 MicroBeads and the autoMACS Pro Separator. Enriched CD34⁺ cells had viability greater than 99% and purity above 90% (fig. 2A). Following this standardized cell separation process, $2.7\times10^6 \pm 0.22\times10^6$ CD34⁺ cells were isolated from ~8×10^8 cord blood MNCs allowing the generation of 50 HIS mice per umbilical cord blood sample on average (fig. 2B–D).

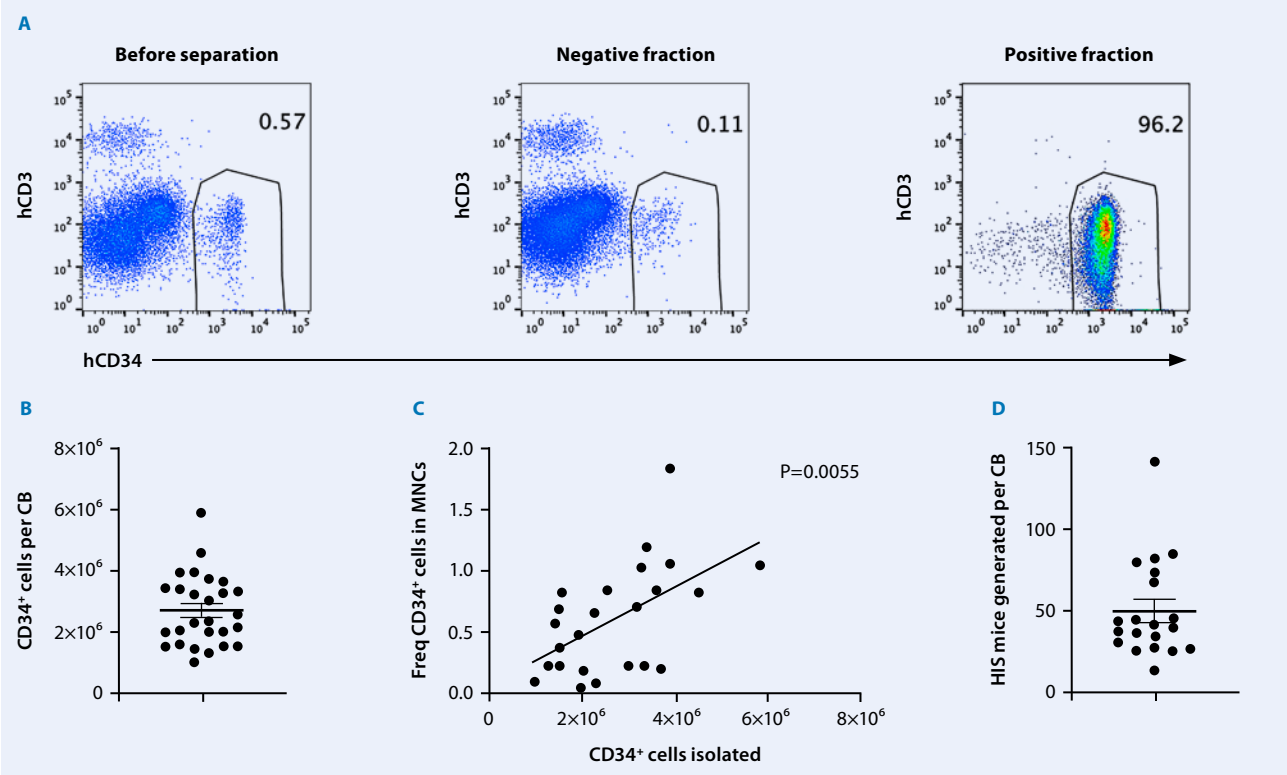

Figure 2: Enrichment of human HSCs from cord blood for creation of HIS mice. (A) Flow cytometric analysis of human CD34⁺ cells from umbilical cord blood before and after magnetic separation with CD34⁺ MicroBeads and the autoMACS Pro Separator. (B) Number of CD34⁺ cells isolated per umbilical cord blood sample; n=27. (C) The frequency of CD34⁺ cells in the unmanipulated cord blood predicted the number of CD34⁺ cells isolated. (D) Number of HIS mice generated per umbilical cord blood sample; n=20.

Testing cancer immunotherapy in HIS mice co-engrafted with human tumors

HIS-BRGS mice bearing TNBC or ACC xenografts, which served as a "hot" and "cold" tumor respectively, were treated with a PD-1 inhibitor and the therapeutic efficacy was evaluated measuring the infiltration of human CD45⁺ cells into the tumors. Immunotherapy treatment highly increased tumor-infiltrating lymphocytes in TNBC CDXs compared to untreated control animals (fig. 3A). On the contrary, no effect on the human infiltration was observed in ACC PDX tumors. This suggests no response to anti-PD-1 treatment (fig. 3B). These data collectively indicate that tumor immune cell infiltration in HIS mice varies by tumor type and this correlates well with clinical data describing "hot" versus "cold" tumors. For further details, refer to Marín-Jiménez *et al.*[5]

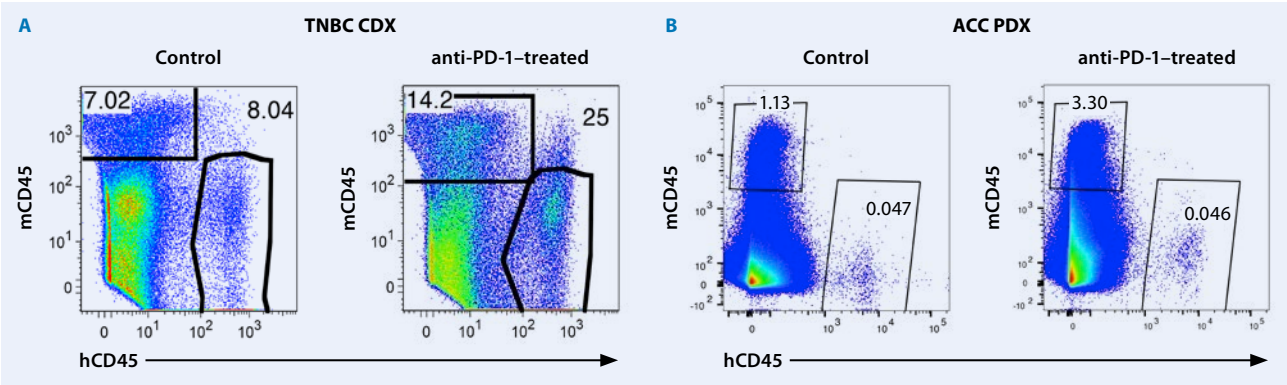

Figure 3: Infiltration of immune cells into human tumors in HIS mice treated with a PD-1 inhibitor. Representative flow cytometric plots showing mouse and human CD45⁺ cells infiltrated into tumors in HIS-BRGS mice implanted with (A) TNBC MDA-MB-231 cells and (B) ACC tumors with and without anti-PD-1 treatment.

Understanding microbiota-immune system interactions in HIS mice

To address the interplay between gut microbiota and human immune system, HIS and control BRGS mice were fed a diet containing the broad-spectrum Septra for two weeks. Feces collected at end of treatment were sequenced for microbiome diversity using 16S rRNA gene sequencing. Microbiome alpha diversity was distinct in HIS mice relative to BRGS non-humanized mice, regardless of antibiotic treatment. The HIS mice showed greater alpha diversity, suggesting the addition of a human immune system influences the gut microbiota. The gut microbiome was distinct in mice reconstituted with a human immune system and unaltered with antibiotic treatment (fig. 4).

These data indicate that HIS mouse models are compatible with microbiome studies and hold great potential to investigate the impact of intestinal microbiome on immune system homeostasis.

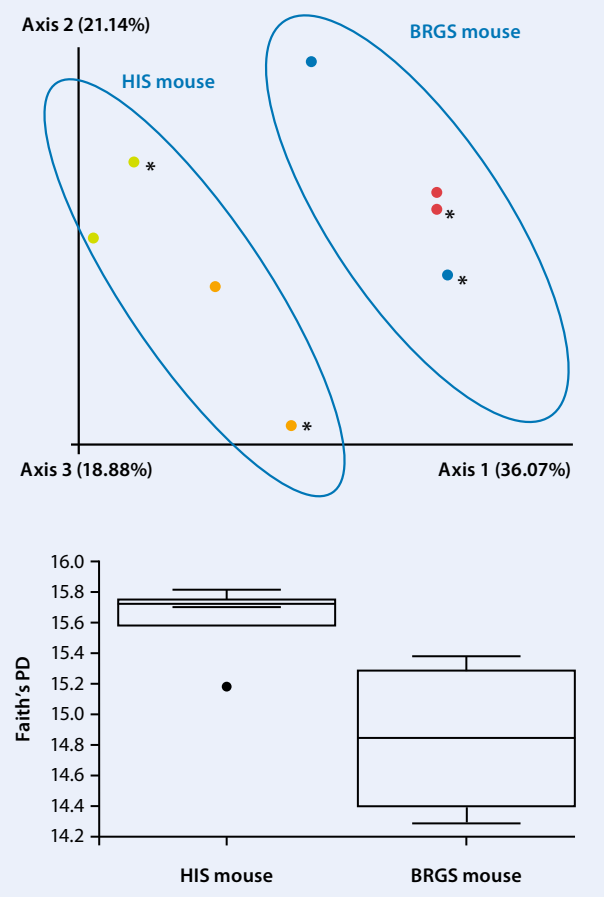

Figure 4: Microbiome alpha diversity in humanized (HIS) and non-humanized (BRGS) mice with and without antibiotic treatment. Colors represent individual mice pre- and post-treatment. Antibiotic (Septra) treated samples are indicated with an asterisk (*).

Conclusion

The present application note shows that MACS® Technology contributes to the successful generation of humanized mouse models and further enhances their applications to advance drug discovery and understand human immunity. Automated magnetic separation of human CD34+ HSCs enables a successful reconstitution of the human immune system in immunocompromised mice, thereby proving that viable and highly pure HSCs retain fully functional potential. The data shown here prove the suitability of the workflow to generate and use HIS mice to test cancer immunotherapy and to investigate the complex interactions between the gut microbiota and human immune system.

Recommended MACS Products

MACS Product	Order no.
CD34 MicroBead Kit, human	130-046-702
CD34 MicroBead Kit UltraPure, human	130-100-453
autoMACS® Pro Separator	130-092-545
Tumor Dissociation Kit, human	130-095-929
gentleMACS™ Octo Dissociator with Heaters	130-096-427

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