Reconstituting the Human Immune System

Better models needed to test checkpoint blockers and other immune therapies for use in patients

Tumor-targeting therapies have long been studied in animal models, particularly human xenografts growing in immunodeficient mice. However, such models—and even mice with patient-derived xenografts (PDX)—are poorly suited for studying checkpoint blockers because many of these drugs act on cells absent in animals lacking a human immune system. "Humanized" models are thus needed to test immune-modulating therapies in a preclinical setting.

To produce such models, researchers are transplanting human $CD34^+$ hematopoietic stem cells (HSC) into immunodeficient mice, coaxing the HSCs to grow and differentiate into functional immune cells. The process is long and laborious, but during a webinar by the American Association for the Advancement of Science, scientists reported progress in reconstituting the human immune system in NOD/SCID gamma (NSG) transgenic mice.

NSG transgenic mice are a widely used host strain for creating humanized mice. In addition to lacking B and T cells and having impaired innate immunity, these mice have a mutated IL2 receptor γ chain gene, which blocks development of natural killer cells. Natural killer cells are generally to blame for tissue rejection, a common problem with immune reconstitution procedures.

After HSCs are transplanted into an immunodeficient NSG mouse, it takes months for them to develop into functional immune cells. Researchers are taking multiple approaches to stimulate earlier and more sustained immune reconstitution. For example, they have created a new mouse strain, NSG-SGM3, by transgenically expressing human cytokines—stem cell factor, granulocyte-macrophage colony-stimulating factor, and IL3—in the NSG mouse. Compared with humanized NSG mice, immune reconstitution occurs earlier and more robustly in the NSG-SGM3 model.

Renata Stripecke, PhD, of Hannover Medical School in Germany, is using a different approach to enhance immune reconstitution. Rather than supplying human cytokines through additional transgenes, her lab injects the host mouse with human dendritic cells. These cells are professional antigen-presenting cells, and also secrete cytokines that promote activation and differentiation of T and B cells. Stripecke produces genetically reprogrammed dendritic cells in the lab by culturing $CD14^+$ monocyte precursors and using lentiviral vectors to induce expression of various cytokines and antigens. The dendritic cells survive 3 to 4 weeks in an immunodeficient mouse, Stripecke says, so the host needs a monthly boost of cryopreserved dendritic cells.

"With this protocol, the host mouse produces mature, functional T cells in 15 to 20 weeks," Stripecke says. If dendritic cells are not used, it takes 20 to 30 weeks for T cells to develop.

Jennifer Torrance, The Jackson Laboratory

NSG transgenic mice are widely used to create humanized mouse models for immunotherapy research.

As methods for creating mice with human immune systems have improved, scientists have begun using them to test checkpoint inhibitors. James Keck, PhD, of The Jackson Laboratory in Sacramento, CA, described a proof-of-concept study examining the effectiveness of pembrolizumab (Keytruda; Merck) with and without docetaxel in humanized NSG mice engrafted with the lung LG1306 PDX tumor. Analyzed 3 to 4 weeks after treatment, mice that received pembrolizumab had slower tumor growth than saline-treated controls, and the slowdown was even more pronounced in mice treated with a pembrolizumab–docetaxel combination.

One concern was the considerable variability in the animals' responses. While eight of 10 mice clearly benefited from the drug, two showed poor responses, which created large statistical deviance for the pembrolizumab group as a whole. "Every mouse is like an individual," says Keck, noting that PDXs are heterogeneous tumors that produce variable responses after being transplanted.

Despite clear progress, some experts remain cautious about using humanized mice to test cancer immunotherapies. Normally, T cells develop in the thymus through a careful selection process that weeds out dangerous self-reactive cells and promotes the development of cells specific to foreign antigens. However, because NSG mice lack a thymus, the T-cell repertoire that develops in these animals after HSC engraftment may be abnormal, says Norman Sharpless, MD, director of the University of North Carolina (UNC) Lineberger Comprehensive Cancer Center in Chapel Hill. Plus, the mice "reconstitute at different rates and to different degrees... and the transplanted human tumors take at variable rates," Sharpless says. "By the time you get reconstituted mice with an established human tumor, there is a lot of heterogeneity in cohorts that are pretty small."

While humanized mice should, in theory, allow for studies of responses to immune-based therapies, PDX models "have more genomic diversity and, as such, may be more effective for investigating predictive biomarkers of response to therapy," says William Kim, MD, co-director of UNC's Mouse Phase 1 Unit. In contrast, genetically engineered models are best suited for probing specific genes and their effects on tumor biology and phenotype. "Like much of science, the best model depends on the question being asked," Kim says. —*Esther Landhuis* ■

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