

A Vascular Niche for Boosted Blood Stem Cell Production

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Many patients with hematopoietic disease rely on transplantation of hematopoietic stem/progenitor cells (HSPC) for a cure. Regardless of transplant source—healthy donors or the patients' own genetically modified cells—treatment success depends on proper engraftment. Allogeneic transplantation has a good track record, but is limited by donor availability and adverse effects such as graft-versus-host disease. The advent of autologous stem cell gene therapies introduced a promising solution to both issues; however, the production of long-term engrafting HSPCs must be improved for treatments to be clinically realistic.

How can this be achieved? First of all, different kinds of HSPCs have varied self-renewal, differentiation, and engraftment potential. Pluripotent stem cells (PSC) can propagate indefinitely and may be directed to make many cell types in the human body, and could therefore be used as an unlimited HSPC source. Hematopoietic multipotent progenitor cells (MPP) are derived from PSCs and share the basic blood stem cell features. However, clinical translation of blood therapeutics utilizing human PSC-derived MPPs has been hampered by inadequate engraftment and poor proliferation in mouse models, prompting exploration of the underlying mechanisms behind blood cell development. Findings indicate that endothelial cells (EC) are key players for development and maintenance of HSPCs, by forming specialized microenvironments within the bone marrow and other organs, referred to as "vascular niches"—units with both anatomic and functional dimensions that determine the various interactions that influence blood stem cell formation. More specifically, studies of signaling pathways and gene expression in the vascular endothelium suggest an important role for the Notch pathway for boosting the proliferation of human long-term MPPs—a possible lead to improved HSPC production.

These findings inspired Drs. Jennifer Gori and Hans-Peter Kiem from the Clinical Research Division to hypothesize that EC-induced Notch ligands may stimulate blood stem cell specification, and that an artificial *ex vivo* vascular niche could increase the production of long-term blood MPPs from PSC precursors. The idea was tested by generating induced PSCs directly from mature macaque cells, allowing evaluation of PSC-derived MPP engraftment and proliferation in mice. Published in *Journal of Clinical Investigation*, the results showed a clear benefit of EC-induced PSC-MPPs over cytokine-induced control cells for initial engraftment and retainment over time. *In vivo* selection using low-

dose chemotherapy of gene-modified PSC-MPPs—genetically modified to be chemotherapy-resistant—further increased the levels, tripling the bone marrow engraftment of EC-induced progenitor cells compared with hematopoietic cells that were exposed to cytokines alone. In addition, cells produced through the artificial vascular niche exhibited desired stem cell-associated traits such as multipotency and self-renewal.

To shed further light on the vascular niche functionality the investigators also studied differentiation and engraftment of human embryonic stem cells (hESC) with and without EC-mediated activation of the Notch pathway. The comprehensive evidence indicated a key role for the EC-associated Notch ligands JAG1 and DLL4 in the differentiation of PSCs, and that PSC-MPPs generated in the context of a vascular niche were significantly more active, both *ex vivo* and *in vivo*. "It has been well established that the vascular niche is the initial site of blood development in the embryo. Here, we show that EC-mediated activation of the Notch pathway in primitive blood stem cells facilitates the maturation and possibly the long-term engraftment of stem cells produced from PSCs," explained Dr. Gori.

The studies demonstrate the potential for scaled production of functional HSPCs via reprogramming of patient cells, followed by growth factor-mediated induction of blood stem cell precursor formation, and culture of these precursors on endothelial cells for their maturation to an HSPC-like state—an important technical advance. "Being able to generate large numbers of hematopoietic stem cells has significant implications for autologous transplantation for patients with a number of hematologic malignancies," Dr. Kiem explained, adding that the discoveries apply also to gene therapy. "Now we can generate stem cells, correct them *ex vivo*, eg. for hemoglobinopathies, then expand corrected autologous stem cells and infuse them back into the patient." What's more, transplantation of larger amounts of corrected cells should require less pre-infusion chemo- or radiation therapy for successful engraftment.

Dr. Kiem and colleagues are now adapting the methodology for generation of sufficient amounts of cells for clinical applications, and companies are working on scaling up the technology. Some questions remain, for instance whether the ECs need to be eliminated before infusion or if they can be irradiated and die off in the patient after ensuring engraftment of the expanded stem cells. Drs. Gori and Kiem do not hold all the answers yet, but the investigators are one important step closer to generating long-term HSPCs—a goal that could benefit many who suffer from hematologic disease.

[Gori JL, Butler JM, Chan Y-Y, Chandrasekaran D, Poulos MG, Ginsberg M, Nolan DJ, Elemento O, Wood BL, Adair JE, Rafii S, Kiem H-P.](#) 2015. Vascular niche promotes hematopoietic multipotent progenitor formation from pluripotent stem cells. *J Clin Invest.* 125(3):1243-54.

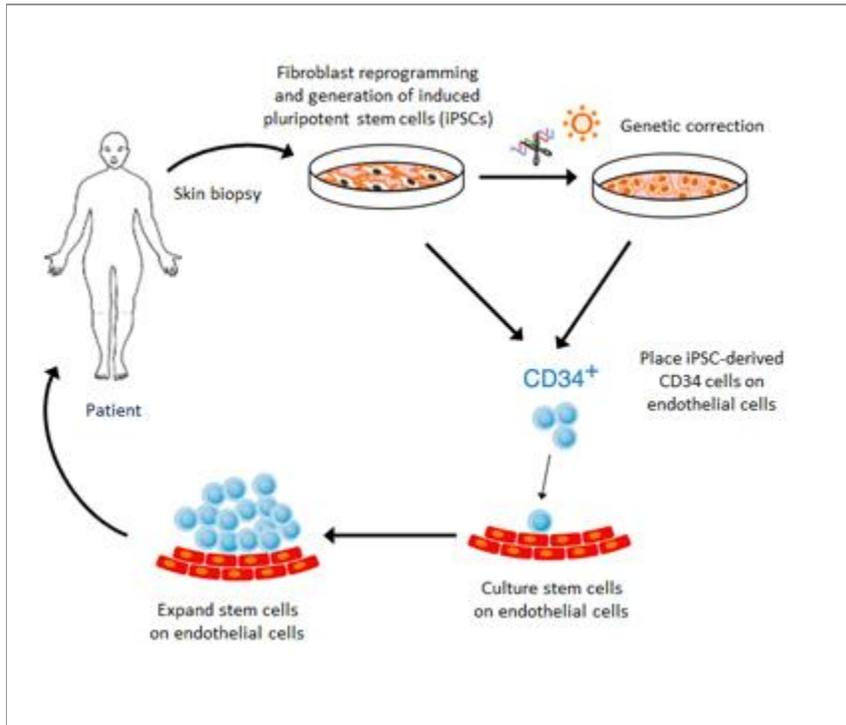


Image provided by Dr. Hans-Peter Kiem

Expansion of hematopoietic stem cells using an artificial ex vivo vascular niche. Patient cells (fibroblasts or other somatic cells including lymphocytes) are reprogrammed to generate induced pluripotent stem cells, which differentiate into CD34-expressing progenitors (with or without additional gene therapy). The CD34⁺ cells are then cultured on endothelial cells that express JAG1 and DLL4, boosting the expansion to sufficient amounts for autologous transplantation back into the patient, to replace diseased blood cells.