bortezomib. It is not immediately apparent which of these is better than conventional treatment. This uncertainty together with the large number of drugs to test will likely sound the death knell for the conventional phase 3 trial, especially when utilizing conventional hazard ratios. Phase 3 trials are ill-suited for a disease as heterogeneous as AML. Both in Europe and recently in US cooperative groups, smaller comparative trials have been explored, under the assumption that the worst false-negative results when a drug is not studied at all.[10] In this way, as in the introduction of qualitatively distinct drugs such as lenalidomide, clinical research in AML is truly in ferment.

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● ● ● **HEMATOPOIESIS & STEM CELLS**

Comment on Leung et al, page 1840

CD9 phones home with a TEM of its own

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Tetraspanins are small molecule proteins known to impact cellular migration and signaling. In this issue of *Blood*, Leung and colleagues uncover a novel function of the tetraspanin molecule CD9 as a potential mediator of $CD34^+$ cell homing.[1]

Signaling between CXCR4 and its ligand—stromal-derived factor 1 (SDF-1/CXCR12)—is clearly important in hematopoietic stem cell (HSC) homing and engraftment, but the precise mediators involved are unclear. Recently, attempts to exploit this axis for benefit in the clinic have led to the development of the CXCR4 inhibitor, plerixafor,[2] and several other similar agents are in development. Given the catastrophe of engraftment failure in hematopoietic stem cell transplantation (HSCT), and the increased rates of such an outcome with cord blood HSCT in adults, attempts to maximize engraftment by enhancing homing are tantalizing.

The use of microarray screening technology in the elucidation of essential mechanistic targets led to many exciting discoveries in the past decade; in this issue, Leung and colleagues explore the role of CD9, a tetraspanin protein found in a screen, as one in a series of genes preferentially induced by SDF-1 in human $CD34^+$ cord blood cells.

Cell-surface proteins of the tetraspanin family have 4 transmembrane domains, intracellular N and C termini, and 2 extracellular domains. Tetraspanins are thought to act as scaffold proteins: multimolecular organizers which anchor proteins to one area of the cell membrane thereby forming structures known as tetraspanin-enriched microdomains (TEMs).[3] Tetraspanins are therefore often considered molecular facilitators modulating the activities of their associated molecules depending upon the TEM composition. Interestingly, a TEM formed by CD9 often includes HSC homing proteins B1 integrin,[4] MTI-MMP,[5] and CD26.[6,7]

Leung et al transplanted $CD34^+CD9^-$ cells and whole $CD34^+$ cells (CD9 antibodies used to positively select $CD34^+CD9^+$ cells had neutralizing effect and were not used for this reason) into *NOD.CB.17-Prkdc^{scid}/J* (NOD-Scid) mice, both with and without anti-CD122 antibody. After establishing a clear increase in homing among $CD34^+CD9^+$ cells in the bone marrow and spleen of mice 20 hours after transplantation, the authors elegantly put several known pharmacologic inhibitors of the effectors of the SDF-1/CXCR4 pathway to use to suggest a signaling pathway leading to CD9 expression via activation of the transcription factor *STAT*. Interestingly, phosphatidylinositol 3-kinase was not involved in signaling of CD9 transcription as it is with other factors involved in homing via mediated SDF-1.[8]

Enhancement of stem cell engraftment has significant clinical relevance in the era of cord blood transplantation. Leung and colleagues are the first to demonstrate that CD9 enrichment improves homing of $CD34^+$ cells to the bone marrow in the in vivo xenograft assay. It will be interesting to explore potential relationships between CD9 and other SDF-1–induced proteins—regardless of their association with the CD9 TEM—known to impact HSC homing such as CD44[9] and CD26.[6] And, of course, the question remains open as to the effect this improved homing will have on HSC engraftment.

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● ● ● ● IMMUNOBIOLOGY

Comment on Wang et al, page 1888

Fitness without exhaustion

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Next-generation adoptive leukemia immunotherapy will likely be based on injection of T cells targeted to specific antigens (Ag). This approach is currently limited by our inability to generate sufficient numbers of Ag-specific T cells that can survive and proliferate in vivo. In this issue of *Blood*, Wang and colleagues demonstrate that central memory T cells can generate ex vivo Ag-specific T cells that thrive well after in vivo transfer.[1]

Cure of hematologic malignancies after standard allogeneic hematopoietic cell transplantation (AHCT) is due mainly to the so-called graft-versus-leukemia (GVL) effect, that is, eradication of host neoplastic cells by donor T cells. The allogeneic GVL effect represents, by far, the most successful form of cancer immunotherapy in humans. It is initiated by T cells mostly found in the naive compartment, which recognize minor histocompatibility Ags (MiHAs) and also perhapsleukemia-associated Ags.[2,3]

GVL induction has practically not evolved over the last 2 decades. Accordingly, despite its great paradigmatic and clinical relevance, the allogeneic GVL effect remains a quite rudimentary form of leukemia immunotherapy. It still involves injection of unselected donor lymphocytes that have not been primed against their target Ags. This current approach is fraught with 2 major limitations. First, injection of polyspecific allogeneic T cells lacks specificity and is therefore highly toxic: these T cells react against several host MiHAs, resulting in graft-versus-host disease (GVHD) in 60% of recipients. Second, naive unprimed T cells induce only an attenuated form of GVL reaction because, in contrast to pre-activated T cells, they can become tolerant after encounter with tumor cells. Experimental mouse models have revealed that far superior results could be achieved by adoptive immunotherapy with preactivated Ag-specific T cells: primed T cells targeted to a single MiHA have cured leukemia and melanoma without causing any toxicity to the host.[4,5] The outcome depended on 2 T-cell effector mechanisms: direct killing of neoplastic cells by granule exocytosis and inhibition of angiogenesis by interferon γ.[4,5]

If leukemia immunotherapy with activated Ag-specific T cells holds such great promises, what is the problem with its application in humans? The mean frequency of Ag-specific T cells in the preimmune human T-cell repertoire is approximately 10^{-5} and at least 10^8 to 10^9 Ag-specific T cells are presumably required for successful treatment.[6,7] Therefore, procurement of sufficient numbers of T cells for immunotherapy requires massive expansion of the few Ag-specific T cells present in a donor's peripheral blood. Proof-of-principle studies in mice resorted to a trick that cannot be used in humans: expansion of Ag-specific T cells was performed in vivo by immunizing the donor against the target Ag.[4,5] Because donor immunization would raise major ethical issues in humans, we are left with one possibility: to proceed with ex vivo expansion of Ag-specific T cells. That would be theoretically feasible if we knew how to generate self-renewing memory T cells. While such

memory T cells having stem cell–like self-renewal potential exist in vivo,[8-10] we ignore how to generate them ex vivo. Current methods for ex vivo expansion of Ag-specific T cells yield poorly functional exhausted T cells that rapidly disappear after in vivo transfer. Nonetheless, we know that naturally occurring memory stem cells reside in the pool of central memory T cells (T_{CM}). Using an instructive mouse model, Wang et al found that after massive ex vivo expansion, the progeny of human T_{CM} established a persistent reservoir of functional T cells in vivo.[1] Accordingly, they now propose a novel strategy for generating therapeutically relevant numbers of Ag-specific T cells: introduction of genes encoding tumor-specific receptors into T_{CM} followed by ex vivo expansion and adoptive transfer. It is of course difficult to predict the merit of that approach, but further studies along those lines will be instructive and are eagerly awaited.

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