Comment on Copelan et al, page 3863, and on Bredeson et al, page 3871

**Busulfan or TBI: answer to an age-old question**

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In this issue of Blood, authors of 2 articles have compared busulfan with total body irradiation (TBI) in preparative regimens for hematopoietic transplantation as treatment of acute myeloid leukemia (AML).1,2

Myeloablative preparative regimens are designed to eradicate the leukemia and provide sufficient immunosuppression to prevent graft rejection. The optimal regimen for patients with AML is controversial. Cyclophosphamide (Cy) combined with either TBI3 or busulfan (Bu)4,5 have been widely used. A randomized study published in 1992 showed superior results with the Cy–TBI compared with Bu-Cy using the oral busulfan formulation available at that time.6 Oral busulfan is erratically absorbed, resulting in broad variation in blood levels. Busulfan systemic exposure is related to toxicities, disease control, and treatment-related mortality.7-9 An intravenous busulfan formulation has subsequently been developed, which provides more consistent pharmacokinetics and reliable dosing10; it may also avoid first-pass hepatotoxicity related to oral dosing. Intravenous busulfan has largely replaced the oral formulation in preparative regimens for hematopoietic transplantation.

These 2 articles directly compare busulfan and TBI for hematopoietic transplantation as treatment of AML in the modern era. Copelan et al performed a retrospective review of 1230 patients with AML in a complete remission reported to the Center for International Blood and Marrow Transplantation Research receiving Cy–TBI or Bu-Cy with either the oral or intravenous busulfan formulation between 2000 and 2006.1 By multivariate analysis, they report significantly less nonrelapse mortality and relapse after 1 year posttransplant and better leukemia-free and overall survival for those receiving intravenous, but not oral, Bu-Cy compared with Cy-TBI. Similar conclusions applied to TBI given with standard fractionation or in higher doses. Thus, these data indicate reduced toxicity and improved survival with the intravenous Bu-Cy regimen compared with Cy-TBI. Leukemia-free survival and survival is shown in the figure.

Bredeson et al performed a second “prospective” nonrandomized cohort study between 2009 and 2011, testing the noninferiority of survival with intravenous busulfan vs TBI-based myeloablative preparative regimens for hematopoietic transplantation for myeloid malignancies.2 A total of 1483 patients were enrolled; most had AML. Cohorts were balanced for prognostic factors impacting outcome. For AML patients, 2-year survival was 57% with busulfan and 46% with TBI-based regimens; \( P = .003 \). The only concern of note was a higher incidence of hepatic venoocclusive disease for Bu (5%) than with TBI (1%). There were no differences in progression-free survival and graft-versus-host disease. The combination of busulfan and fludarabine has been studied as a potentially less toxic alternative to busulfan-cyclophosphamide.11,12 The Bredeson study also compared patients receiving busulfan-fludarabine vs busulfan-cyclophosphamide; there was no difference in outcomes with these preparative regimens.

Together, these 2 studies indicate improved survival in patients receiving a preparative regimen using intravenous busulfan compared with TBI for myeloablative hematopoietic transplantation as treatment of AML. Ideally, this should be confirmed by a randomized controlled trial, but a similarly sized randomized trial is not feasible. The available data indicate that, compared with myeloablative TBI, a preparative regimen using intravenous busulfan is relatively safe and effective for treatment of AML, and thus it should be considered a standard of care for allogeneic hematopoietic transplantation in these patients.

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Comment on Aranguren et al, page 3982

**New specs for arteriovenous identity**

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In this issue of Blood, Aranguren et al provide new insights into the molecular mechanisms that determine the identity of human endothelial cells: ie, will they line arteries or veins? The findings have implications in our understanding of vascular disease and in the design of vascular-specific therapies and tissue engineering.

Endothelial cells lining arteries and veins arise from mesoderm-derived angioblasts in the embryo. The common origin of these endothelial cell populations belies the fact that arteries and veins have distinct structural features, properties, and functions. The Greek anatomist Erasistratus is credited with being the first to distinguish these 2 vessel types during the third century BCE. Even though he believed that they carry air, his insights have spurred generations of scientists, including Galen (second century AD), Harvey (17th century AD), and, most recently, Aranguren et al to attempt to delineate the unique “signatures” of arteries and veins and how they evolve.

What dictates whether an angioblast will emerge as an artery or vein? The complex molecular mechanisms underlying arteriovenous (AV) specification, particularly in the embryo, have been studied in many species. Although there are differences, all support the notion that AV specification is driven primarily by a cell-intrinsic program that initially occurs independent of blood flow. Mesoderm-derived angioblasts that form the primitive vascular plexus are already fated to be either arterial or venous. Notch and Wnt/β-catenin pathways are currently believed to mainly converge with induction of Notch signaling and consequent upregulation of transcription factor effectors, such as HEY2. These promote arterial endothelial specification, with increased expression of neuropilin-1 (Nrp-1) and ephrinB2, suppression of venous endothelial markers EphB4 and Nrp-2, and release of factors that encourage growth, differentiation, and recruitment of vascular smooth muscle cells. This apparent default pathway to an arterial fate is checked by COUP-TFI1, which suppresses Nrp-1 and Notch, thereby promoting and/or maintaining a venous endothelial identity.

In spite of these insights, the genetic, epigenetic, and environmental factors that determine AV fate remain incompletely understood. Efforts to more readily decipher the underlying molecular mechanisms increased as it became easier to culture human endothelial cells. Although this technology is attractive, the question of whether cultured cells can recapitulate the in vivo situation has been examined only to a limited extent. Aranguren et al are the first to use an unbiased genome-wide approach to discriminate endothelial cells that are freshly isolated from arteries vs veins, in conjunction with an analysis of the impact of in vitro cell culture on AV specification. Using cultured endothelial cells from multiple adult vascular beds, they first showed that gene expression profiles could not be distinguished based on arterial or venous origin. They then demonstrated that freshly isolated human umbilical vein and arterial endothelial cells yielded an “AV-fresh” gene profile that could be used to reliably separate venous from arterial endothelial cells. This distinction was entirely erased when the cells were cultured for even a short time (see figure).

From the AV-fresh gene profile, Aranguren et al went on to characterize the function of 8 arterial endothelial-specific transcription factors, 6 of which have not been implicated previously in AV specification. Collectively, these factors were more effective than any single factor, including HEY2, at restoring and sustaining the arterial endothelial fingerprint of cultured cells. Even though this novel combination of transcription factors could only restore ~75% of the arterial fingerprint in vitro, it did impart an arterial phenotype to human umbilical vein endothelial cells in an in vivo model.

There are several novel and important implications of these studies. The findings highlight the importance of considering how rapidly and dramatically in vitro culture alters the genetic and functional properties of cells. As implied in this report, it is likely that the molecular signatures of freshly isolated endothelial cells from a vascular bed are closer to what exists in vivo, particularly as compared with samples after days or weeks in culture. It should be expected that there
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