Dry Beer, Ice Beer and ISHAGE: Evolution of Beer and CD34+ Cell Enumeration.

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To address a major clinical issue of when to perform apheresis on cancer patients undergoing cytokine-induced mobilization of their CD34+ cells for autologous bone marrow transplantation, we developed in 1994 a simple, highly sensitive, rapid and specific two-color (CD34PE/CD45FITC) flow cytometric procedure to measure these cells down to the 0.02%. This was the first flow method based on the biology of CD34+ cells, the structural characteristics of the CD34 molecule, and the biochemical characteristics of the epitopes detected by various classes of CD34 (and CD45) antibodies. This method could be performed on a variety of flow cytometric platforms and on various sources of hematopoietic stem cells (bone marrow, mobilized peripheral blood, cord blood etc). In 1996 this methodology was embodied in a published document (The ISHAGE Guidelines For CD34+ Cell Determination By Flow Cytometry. J Hematother 5: 213, 1996) for ISHAGE (International Society of Hematotherapy and Graft Engineering (pronounced 'ICE-AGE'). To derive and absolute CD34+ cell count in a sample required the additional derivation of the absolute White Blood Cell count from a hematology analyser. Subsequent developments involved the inclusion of the viability dye 7-AAD and fluorescent counting beads converting the original 'two platform' method into a 'single platform' method to determine the absolute viable CD34+ cell count in a blood sample without the need for a hematology analyser (Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE Guidelines. Cytometry (Comm Clin Cytom) 34: 61, 1998).

Enumerating viable CD34+ cells provides critical information to the bone marrow transplant physician. The number of viable CD34+ cells present in the peripheral blood after mobilization with cytokines and/or chemotherapy predicts the 'yield' of CD34+ cells in the apheresis product. Additionally, the number of CD34+ cells collected predicts time

to engraftment after autologous or allogeneic HSC transplantation. The infusion of a minimum of 2-2.5 x 10^6 viable CD34+ cells per kilogram patient weight will generally ensure rapid (10-12 days for neutrophils to 500/uL) and sustained engraftment in the auto-transplant setting.

The ISHAGE Guidelines is the most widely used method for the enumeration of viable CD34+ cells in clinical laboratories and several commercial kits are available (Stem-Kit™, Beckman, CD34Count Kit™ Dako, and SCE Kit™, BD Biosciences) based upon these Guidelines (for auto transplants). The ISHAGE methodologies are also embedded in several International and National Guidelines for performing this critical assay, as well as in published monographs for Current Protocols in Cytometry and the American Association of Blood Banks Technical Guidelines.

While manual data acquisition and analysis templates/protocols were developed years ago for a variety of older cytometers equipped with 3 (FACScan BD), 4 (Calibur, BD, Epics XL, Coulter) or 5 (FC500, Beckman) fluorescence defectors (PMTs), the widespread deployment in clinical labs of newer instruments with 6 or 8 PMTs (Canto, Canto II BD) and 8 or 10 PMTs (Navios/Gallio, Beckman), required us to develop equivalent assays that could run on newer computer systems running more modern operating systems/flow cytometry software.

We have thus validated the single platform ISHAGE protocols across instruments with 4 PMTs (Calibur), 5 PMTs (FC500), 8 PMTs (Canto II), and 10 PMTs (Navios) and show that equivalent data is generated regardless of instrument platform/software combination used (T-Test showed no significant difference). For example, analysis of 20 fresh mobilized PB/apheresis samples across FC500 and Navios instruments using Stem-Kit™ yielded virtually identical results with a high correlation coefficient of 0.9995. Manual analysis of another batch of samples stained with the SCE-Kit™ on both Calibur and Canto II cytometers also generated very similar results with correlation coefficient in excess of 0.99. While addition of a CD3PC5 conjugate in place of 7-AAD allowed the simultaneous enumeration of CD3+ cells in fresh donor samples used in the allo-transplant setting, the increasing use of matched unrelated donor transplants in which samples are often shipped internationally required the incorporation of both 7-AAD and CD3. Thus we have developed 4-color variants of the single platform ISHAGE

methodology that can simultaneously measure not just absolute viable CD34+ and CD45+ cell content but also the absolute viable CD3+ cell content.
We have validated the auto and ‘allo’ variants of the single platform ISHAGE protocol across multiple platforms.
Finally, it is not uncommon for allo-transplant recipients to require donor lymphocyte infusions (DLI) and the allo ISHAGE variant provides a rapid and validated means to accurately determine the number of viable CD3+ cells to be infused in this context. The allo assay can also be used to measure the CD34+ cell purity and residual contaminating viable CD3+ cells in CD34+ cell-selected samples.