Editorial

Issue Highlights—May 2012

PROGNOSTIC SIGNIFICANCE OF LEUKEMIC SUBCLONES IDENTIFIED BY MULTIPARAMETER FLOW CYTOMETRY

The paper in this issue by Johnsen et al. "Clinical impact of leukemic blast heterogeneity at diagnosis in cytogenetic intermediate-risk acute myeloid leukaemia," deals with an important issue that is central to the role played by persistent leukemic cells in acute myeloid leukemia.

Specifically, the heterogeneity of leukemic blasts is thought to have significant relationship to the prognosis of the disease; this is demonstrated by numerous studies recently published in our journal at the cytogenetic and molecular level as well as by documented immunophenotypic variations found in the malignant cells (1,2,3,4,5). Interestingly, there is growing evidence that malignant cells and stem cells traffic similarly and may share a common niche (6). Johnsen's paper demonstrates that leukemic cancer stem cells share surface molecules in common with normal stem cells, and both cell types are characterized by high self-renewal and pluripotency. The persistence of leukemic cancer stem cells is considered to be essential in the maintenance of acute myeloid leukemia and its relapse. Recent advances in this area focus on the development of new drugs to eradicate leukemic stem cells responsible for sustaining leukemic growth. It is also clear that for improved treatment results more knowledge about malignant stem cells is required, and further studies are needed to clarify this issue. A flow cytometric protocol enabling discrimination of myeloid leukemia stem cells from their normal counterpart is essential in this challenging field, which these authors have provided greater insight into.

CD300A SUPPRESSES BASOPHIL DEGRANULATION

Although not often identified in great numbers in the normal human circulation, the critical role of basophils has become an increasingly active area of study for the part they play in both allergy and malignancy and is an area ripe for study by cytometry (7,8,9). Our Clinical Cytometry Journal readership has been most fortunate in benefitting from the excellent contributions of a very active and excellent group of investigators at the Antwerp University Hospital who have been focusing a great deal of effort in this area (10,11). Normal basophil activity and degranulation is a highly regulated process that depends on the interaction of competing activating and inhibitory signals. In this issue, the contribution "CD300a is expressed on human basophils and seems to inhibit IgE/Fc·RI-dependent anaphylactic degranulation," by Verwiej et al. begins to unravel those mechanisms that lead to basophil inhibitory signals related to the CD300a receptor. The ITM-containing CD300a is not specific to the basophil, but is also found on numerous leukocytes and dendritic cells; how CD300a impacts all of these various cell-types remains to be fully elucidated.

THE POWER OF A POLYCHROMATIC APPROACH TO PAUCI-CELLULAR CEREBRAL SPINAL FLUID SPECIMENS

Flow cytometry has been shown to be a very sensitive and effective modality for the characterization of cells found in cerebrospinal fluid (12,13,14). When very few cells are available for immunophenotypic analysis, polychromatic flow cytometry truly provides benefit to clinical care. Such analysis is more statistically robust and medically relevant because increased numbers of patient cells can be interrogated by more monoclonal antibodies, at the same time. In this issue Alessandra et al. demonstrate this principle expertly in a 10-antibody single-tube approach for the analysis of cerebrospinal fluid for B-cell malignancy. In this study, specimens that had fewer than 10 cells/ml were compared by conventional cytology to a polychromatic approach and an increased sensitivity of the cytometric approach was observed.

THE PROGNOSTIC RELEVANCE OF CD8 CO-EXPRESSION ON CHRONIC LYMPHOCYTIC LEUKEMIA

Malignant cells may express cell surface antigens which are not characteristic of their cell type. Such observations have been thought variously as combinations of either our incomplete understanding of normal differentiation states, lineage "infidelity" or perhaps perturbations at the stem-cell level that manifest themselves in the further differentiated neoplastic cell. While such uncharacteristic expression is common in less well-differentiated hematopoietic malignancies such as acute myeloid and lymphoblastic leukemia, it is not often observed in chronic lymphocytic leukemia. Most recent attention in chronic lymphocytic leukemia has been focused specifically on improvements in the prognostic significance of ZAP-70 expression (15,16) and the relevance of monoclonal B-cell proliferation to chronic lymphocytic leukemia, so much so that special issues of our Journal have been previously fully devoted to these topics (17,18). Those involved clinical diagnostics for sufficient years have noticed that infrequently, CD8 was co-expressed on chronic lymphocytic leukemia cells. It was speculated that the ephemeral incidence of such expression might relate to simply 'not looking' systematically for CD8 expression on the malignancy

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(19). In this issue, in evaluating 5,523 patients, Kern et al. have done an excellent job of illuminating the relationship between CD8 expression, IGHV status, ZAP-70 and CD38 co-expression and how these all inter-relate to the prognostic status of the patient.

PHOSPHO-FLOW CYTOMETRY FOR THERAPEUTIC DRUG MONITORING

The mammalian target of rapamycin (mTOR) pathway plays a critical role in the regulation of mRNA translation and is thus involved in cell cycle progression, proliferation, angiogenesis, and cellular survival pathways. Inhibitors of the mTOR pathway, such as sirolimus, are used as immunosuppresives clinically in combinations with cyclosporine, tacrolimus, mycophenolate acid and corticosteroids to prevent the rejection of transplanted solid organs. In the administration of all such therapies, understanding the pharmacodynamics is critical to ascertain the appropriate dosages of the therapies administered. In the very exciting study "Assay validation of phosphorylated S6 ribosomal protein for a pharmacodynamic monitoring of mTOR-inhibitors in peripheral human blood," Dieterlen et al. examined the presence of phosphorylated S6 ribosomal protein (p-S6RP) in T-cells to measure the impact of the immunosuppressive effects of sirolimus therapy. These investigators validated an *in vitro* test and found that phospho-flow cytometry offers an excellent way to identify functionally activated T-cell populations by the presence or absence of phosphorylated proteins within those cells.

CELL SIGNALING INFORMATION IN AML CAN BE STUDIED FROM CIRCULATING BLOOD SPECIMENS

By combining multiparameter flow cytometry with single cell nucleotide profiling, a dynamic and quantitative view of intracellular functional events over time can be appreciated. This technique is useful as a way to understand the results of therapy or relapse risks in AML. In this issue Cesano et al. in the article "Functional pathway analysis in acute myeloid leukemia using single cell network profiling (SCNP) assay: effect of specimen source (bone marrow or peripheral blood) on assay readouts," compared peripheral blood to bone marrow from 46 patients with AML and determined that blood proteomic results correlated well with those obtained from the marrow. These results suggest that blood specimens can be informative to the direction of therapy when obtaining bone marrow is not clinically justifiable.

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