Chapter 10

CLINICAL FLOW CYTOMETRY
A Transition in Utilization

Charles L. Goolsby, Mary Paniagua, Laura Marszalek

Department of Pathology (CLG), Robert H. Lurie Comprehensive Cancer Center (CLG and MP), Northwestern University Feinberg School of Medicine, and Northwestern Memorial Hospital (LM), Chicago, Illinois

1. INTRODUCTION

Over the last 25 years, flow cytometry analysis of hematopoietic malignancies has evolved dramatically. In the early days, single color analysis, or one antigen at a time, on ficoll-hypaque separated cells was the standard approach. Thus, co-expression of antigens on cells, a hallmark of current practice, could only be inferred by comparing numbers of cells positive for each antigen. Density gradient preparation of the cells carried with it the potential for either loss or enrichment of specific cell populations that was variable from sample to sample. These technical limitations limited the sensitivity and specificity of leukemia/lymphoma immunophenotyping in those early days. Certainly, even with these limitations, however, these data were still very useful as an adjunct to diagnosis, although at the time, seldom were they integrated into the primary diagnostic package. Most frequently, the immunophenotypic results were issued as a separate document, frequently days after the primary diagnostic pathology report.

In the intervening years since those early beginnings, there has been steady development of reagents and instrumentation. We have seen not only dramatic increases in the numbers of highly specific monoclonal antibodies but, particularly recently, in the numbers of fluorescent dyes available as well. The standard of practice is now routine four color immunophenotyping with many laboratories moving to five, six, seven or more colors. For the most part, density gradient preparation of cells for these analyses is a thing
of the past. These changes have dramatically increased the sensitivity and specificity of these assays. The analyses have become the preferred method of lineage determination in acute leukemias when morphology or special stains are unable to make that assessment.\textsuperscript{1,2} Sub-classification of the B cell chronic lymphoproliferative disorders now depends heavily on immunophenotypic data,\textsuperscript{2} and cytometric analyses provide a sensitive method of detection of clonal B cell populations.\textsuperscript{2} Routinely, laboratories can detect malignant cell populations at significantly less than 1\% of the total cells (frequently less than 0.1\%) and in some cases can approach RT-PCR levels of sensitivity. In addition, it is now accepted that careful and thorough correlation of these data with other pathology findings, cytogenetics, and molecular analyses is critical for correct interpretation and maximizing the appropriate utilization of these data.\textsuperscript{1,2} As a result, in most institutions, immunophenotyping has become part of the primary diagnostic work-up, ideally incorporated into the primary diagnostic reports. In addition, to continuing to serve an increasingly important adjunctive role in diagnosis, in some instances, immunophenotyping provides important prognostic information as well, for example CD38 expression in HIV,\textsuperscript{3} CD38 expression in B-CLL,\textsuperscript{4,6} or Zap70 expression levels in B-CLL.\textsuperscript{7} Although not the topic of this chapter, the reader is referred to any of a number of excellent reviews on the diagnostic and prognostic uses of these data for more details.\textsuperscript{1,2} It is worth noting that the role as an adjunct to diagnosis continues to evolve with new AML sub-classifications based on signal pathway responses to growth factors being proposed.\textsuperscript{8} This is a very attractive concept in that these measurements will most likely reflect at the functional level abnormalities that are the result of disease characteristic translocations and genetic abnormalities. Perhaps most exciting is that they may then directly tie to new, and emerging, “targeted” therapies directed at these abnormal signaling events that in many instances have lead to the dysregulated proliferation and/or apoptosis that drive the disease process.

2. HISTORICAL PERSPECTIVE

In addition to its utility as an adjunct to diagnosis, exciting new horizons for clinical cytometry are rapidly emerging, and developing, as tools in the therapeutic management of leukemia and lymphoma patients. Increasingly flow cytometric analyses are becoming key in therapeutic decisions and in therapeutic monitoring of patients. The explosion of antigen and ligand directed therapies for a range of hematopoietic malignancies, including anti-CD20,\textsuperscript{9-12} anti-CD22,\textsuperscript{13} anti-CD52,\textsuperscript{11,12,14} anti-CD30,\textsuperscript{15} anti-CD33,\textsuperscript{11,12,16-18} anti-CD45,\textsuperscript{11,17,18} and IL-2,\textsuperscript{19} are having a significant impact on most clinical