



**2013**

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April 1, 2013

Margaret A. Hamburg, MD  
Commissioner, U.S. Food and Drug Administration  
Division of Dockets Management (HFA-305)  
5630 Fishers Lane, Room 1061  
Rockville, MD 20852

Re: Request for Comments: Clinical Flow Cytometry in Hematologic Malignancies  
(Docket No. FDA-2013-N-0046)

Dear Dr. Hamburg:

The American Society of Hematology (ASH) appreciates the opportunity to provide input in the deliberations of the Food and Drug Administration (FDA) regarding clinical flow cytometry (FCM) in hematologic malignancies in response to the Federal Register notice FDA-2013-N-0046 issued on January 24, 2013. ASH applauds the FDA for developing a risk-based regulatory framework for clinical FCM in hematologic malignancies

ASH represents more than 14,000 clinicians and scientists worldwide committed to the study and treatment of blood and blood-related diseases. These diseases encompass malignant hematologic disorders such as leukemia, lymphoma, and multiple myeloma and non-malignant conditions such as sickle cell anemia, thalassemia, aplastic anemia, venous thromboembolism, and hemophilia. In addition, hematologists have been pioneers in the fields of stem cell biology, regenerative medicine, bone marrow transplantation, transfusion medicine, gene therapy, and the development of many drugs for the prevention and treatment of heart attacks and strokes. ASH membership is comprised of basic scientists, physician scientists, physicians, and hematopathologists working in diverse settings, including universities, hospitals and private practices. Hematologists depend on diagnostic flow cytometry as an essential tool for the classification of hematopoietic cells and appropriate diagnosis of hematologic diseases. ASH members and their patients will be directly affected by any type of FDA regulation on flow cytometry as it remains a standard part of the work-up of every hematological disorder and malignancy.

ASH supports the FDA's development of a risk-based regulatory framework that will help bring additional safe and effective products for clinical flow cytometry to market. However, the Society urges the FDA to carefully consider any regulation that would place undue burden on the clinical labs and the physicians diagnosing hematological disease. The Society's specific concerns are outlined below with recommendations highlighted in **bold font**.

**ASH Recommendation 1: FDA should develop a guidance that standardizes some aspects of FCM such as some reagents (e.g., antibody panels) and the software used for interpretation and reporting of results, but the guidance should also recognize the need for flexibility in a rapidly changing field.**

ASH agrees that there is a great need for guidance and regulation of some aspects of FCM and its use in diagnosis of hematologic disease, as there may be laboratories that use lab-developed antibodies for clinical diagnoses without appropriate validation. The Society supports the FDA's efforts to develop a guidance that standardizes some FCM reagents (e.g., antibodies and antibody panels), and software used for interpreting and reporting results. Policies such as Clinical Laboratory Improvement Amendments already in place for other automated laboratory equipment in clinical service labs can potentially inform the FDA as the guidance is being developed. However, the agency needs to recognize that surface expression profiles associated with normal and disease states can change rapidly as the science moves forward, necessitating some flexibility for the rapidly changing field.

For example, the typical flow cytometric findings for AML1/ETO positive AML is expression of the B-lymphoid antigen CD19, expression of CD56, an immature myeloid phenotype and low expression of CD11a. However, it has been recently shown that the same phenotype can be found in but not be associated with AML1/ETO if the blasts also express the T-cell antigen, CD7. This is an important finding since core-binding factor leukemias, such as AML1/ETO positive AMLs, respond very well to high-dose cytosine arabinoside. Thus, there is still a lot to learn when it comes to antigen expression profiles.

ASH also supports standardization efforts that extend to fluorochromes. One of the most important aspects of every antibody panel is the choice of the fluorochrome. For instance, in AML1/ETO positive AML, CD19 is often seen only if the CD19 antibody is conjugated to phycoerythrin or a fluorochrome with similarly high quantum yield. Another example is CD34-conjugated to fluorescein isothiocyanate, which may not be appropriate for use in diagnostic panels for similar reasons.

**ASH Recommendation 2: The FDA should allow the pathologists and hematologists using FCM the flexibility to utilize their expertise for the actual diagnosis and treatment of the patient.**

ASH supports standardizing FCM for diagnosing a hematologic malignancy and informing patient treatment. However, the Society also notes the importance of having FDA develop a guidance that does not inadvertently restrict pathologists and hematologists from utilizing their expertise for the actual diagnosis and treatment of the patient. Diagnosis requires experience and skills from the side of the operator and the interpreter; i.e., the pathologist directing the lab and the hematologist treating the patient.

**ASH Recommendation 3: The FDA should recognize the need for scientific consensus on the role of minimal residual disease (MRD) in patient outcome before developing evidence-based guidance for the use of FCM in MRD for hematologic malignancies.**

ASH recognizes that FCM currently plays a major role in the detection of MRD in hematologic malignancies and that there is a great need for standardized panels to be used for this purpose and guidance for the interpretation of results. The utility of FCM detection of MRD depends in part on whether the same lab is used for initial diagnosis and for MRD detection. In addition, it remains unclear if intervening with a new treatment based upon some threshold of detection of residual disease will likely lead to a better clinical outcome. Thus, there is some potential for harm if FCM results are not correctly interpreted.

It is also important to remember that the clinical significance of any given MRD levels (established by any methodology) may change with the introduction of new treatment regimens, as has been seen with arsenic trioxide versus all-trans retinoic acid in APL. Furthermore, different drugs contained in induction regimens affect MRD differentially, as has been observed with glucocorticoids in ALL. The

increasing use of antibodies to surface antigens in the treatment of leukemias and the associated down-regulation of targeted antigens also needs to be considered in the design of MRD antibody panels and requires some flexibility dependent on treatment regimens.

ASH urges the FDA to recognize that more research is needed on the role of MRD in prediction of patient outcomes. ASH applauds the FDA for hosting the MRD as a Surrogate Endpoint in Acute Lymphoblastic Leukemia Workshop (April 18, 2012); MRD as a Surrogate Endpoint Chronic Lymphocytic Leukemia Workshop (February 27, 2013); and MRD as a Surrogate Endpoint in Acute Myeloid Leukemia Workshop (March 4, 2013) and urges the FDA to use the results of these workshops in informing any potential guidance.

**ASH Recommendation 4: The use of FCM in MRD detection should not be strictly regulated by the FDA before scientific consensus is reached on important intracellular proteins and changing surface proteins as indicators of normal or disease states.**

It is clear that diagnostic flow cytometry is a well-established and essential tool for the appropriate diagnosis and classification of hematopoietic cells and diseases. Studies for many diseases demonstrate that flow-based MRD detection could be an important independent prognosticator of treatment outcome. Nevertheless, the ability to reproducibly and sensitively quantify the detection of the malignant clone that remains following treatment of a disease is an experimental test that is crucial to standardize and “export” to take full advantage of its prognostic value.

The use of FCM in MRD detection depends on how well the population of cells are identified and what fraction of the population contains non-relevant cells. Thus, absolute counting should be emphasized as an index of cells. In addition, more research is needed on mapping the landscape of “normal” differentiation of hematopoietic cells in the bone marrow. Although there are many instances of surface marker expression in leukemias and lymphomas being abnormal, recent studies show that the boundaries of “abnormality” can vary significantly in each disease. In addition, surface marker phenotypes can vary but the underlying biology can be the same (tumor initiating capacity). This raises important questions: 1) which cells outside the bounds of normal constitute a threat? 2) which of those cells are killed by drugs? 3) would it matter if they are killed if they do not have stem cell capacity? 4) given the heterogeneity of marker expression (and near randomness), have sufficient studies been done to “find” cells with unusual marker co-expression profiles and relate them to clinical biology?

Before moving forward with developing any guidance or regulation, the FDA should recognize that the existence of surface markers that denote cells with a given surface phenotype does not mean the intracellular wiring is normal. There remains a need for additional research to interpret stem cell activity of a cell type based on surface markers, as well as MRD being indicative of any set of cell states. For example, there have been multiple reports of intracellular state of the cells migrating independently of surface phenotype. It is important to remember that surface phenotypes are fluid and they are not the final word on biological destiny of the cell. Furthermore, there is increasing evidence for the persistence of leukemic stem cells post therapy which are most likely the source of relapse. To date, antibodies which will recognize those stem cells and allow differentiation between normal and leukemic stem cells are scarce. The picture gets even more complicated with the introduction of pre-leukemic stem cells, which do not differ in their surface antigen profile from normal stem cells though they harbor mutations which may eventually be found in the re-emerging leukemic population.

While ASH recognizes the increased use of FCM in MRD detection and the need for physician community to have guidance on these methods, the FDA should refrain from any regulatory action that

would place an undue burden on flow cytometrists, physicians and their patients until the agency is able to develop recommendations based on the most current science.

The American Society of Hematology (ASH) encourages the FDA to continue to consult with hematologists and hematopathologists as the agency continues to consider regulations in this area. ASH looks forward to working with the FDA to develop a risk-based regulatory framework for clinical flow cytometry that maintains patient access, innovation as well as helps to increase quality of care. ASH will be happy to provide further information and be a resource for the FDA. Please contact ASH Senior Manager for Scientific Affairs, Ulyana V. Desiderio, PhD, at 202-776-0544 or [UDesiderio@hematology.org](mailto:UDesiderio@hematology.org) for any additional information.

Sincerely,

A handwritten signature in black ink, appearing to read "Janis L. Abkowitz". The signature is fluid and cursive, with a large initial "J" and a long, sweeping underline.

Janis L. Abkowitz, MD  
President