

outcome, as shown by the data which demonstrated that over 90% of these were relapse-free after 5 years. The authors found that the results will enable physicians to give more aggressive therapy to high-risk patients, and to give less aggressive and less toxic therapy to low-risk patients.

c. Methodology tip – flow cytometry for assessing minimal residual disease

In the clinical trial of Basso et al. (264) described above, flow cytometry was used for analyzing marrow cells and for generating data used for minimal residual disease analysis. In the analysis of blood cells by flow cytometry, the investigators tagged blood cells with antibodies specific for various markers, also called antigens, that are typically expressed on the surface of leukemic cells of the B cell lineage and leukemic cells of the T cell lineage. As detailed in another publication by Basso et al. (265) the CD19 marker is specific for B cell ALL, and the CD7 marker is specific for T cell ALL. As is the case with all applications of flow cytometry, the antibodies had been modified by covalent attachment of fluorescent dyes to allow detection of tagged cells during passage through the flow cytometer. Details on the technique of flow cytometry, including guidance on choosing antibody tags, as it relates to the leukemias, are provided by Peters and Ansari (266) Uhrmacher et al. (267) and Al-Mawali et al. (268).

d. Using cells acquired after chemotherapy (not before chemotherapy) as a prognostic factor for long-term relapse – the Cilloni study

In a study of adult AML patients, Cilloni et al. (269) measured Wilms' tumor-1 gene (WT1 gene) expression, as a reflection of minimal residual disease (MRD) in patients with AML.

WT1 is not a fusion protein, but it is in a class of proteins that is conventionally known as *tumor antigens*. WT1, which stands for Wilms' tumor gene-1 (not written as Wilm's), encodes a transcription factor. While this gene normally functions to regulate

²⁶⁴ Basso G, Veltroni M, Valsecchi MG, et al. Risk of relapse of childhood acute lymphoblastic leukemia is predicted by flow cytometric measurement of residual disease on day 15 bone marrow. *J Clin Oncol*. 2009;27:5168–5174.

²⁶⁵ Basso G, Buldini B, De Zen L, Orfao A. New methodologic approaches for immunophenotyping acute leukemias. *Haematologica*. 2001;86:675–692.

²⁶⁶ Peters JM, Ansari MQ. Multiparameter flow cytometry in the diagnosis and management of acute leukemia. *Arch Pathol Lab Med*. 2011;135:44–54.

²⁶⁷ Uhrmacher S, Erdfelder F, Kreuzer KA. Flow cytometry and polymerase chain reaction-based analyses of minimal residual disease in chronic lymphocytic leukemia. *Adv Hematol*. 2010 [11 pages].

²⁶⁸ Al-Mawali A, Gillis D, Lewis I. The role of multiparameter flow cytometry for detection of minimal residual disease in acute myeloid leukemia. *Am J Clin Pathol*. 2009;131:16–26.

²⁶⁹ Cilloni D, Renneville A, Hermitte F, et al. Real-time quantitative polymerase chain reaction detection of minimal residual disease by standardized WT1 assay to enhance risk stratification in acute myeloid leukemia: a European LeukemiaNet study. *J Clin Oncol*. 2009;27:5195–5201.