Use of Multiple Technologies to Diagnose, Monitor and Identify MRD in a B-ALL Patient

This case study demonstrates the use of multiple technologies to monitor a patient diagnosed with B-ALL with a CRLF2 rearrangement, a subtype of B-ALL with a possible poor prognosis. After ΔN:™ (difference from normal) Flow Cytometry revealed rare suspicious cells below the level of enumeration of the assay, Fluorescence Activated Cell Sorting (FACS) was used to separate out two populations, CD3+ and CD19+, and FISH (fluorescence in situ hybridization) was then performed on the sorted populations. While HematoLogics was only able to sort 1,500 CD19+ cells, this population was found to be positive for the presence of a CRLF2 rearrangement, confirming MRD. Without sorting for these suspicious cells before FISH analysis, this result would not have been possible. HematoLogics routinely use FACS to increase diagnostic confidence.

CASE STUDY

Clinical History: Patient was diagnosed with B-lymphoblastic leukemia (B-ALL) and monitored over 6 months showing abnormal ΔN:™ flow results of 91%, 35%, 1.7% and 38% abnormal B-lymphoblasts. Diagnostic sample cytogenetics detected loss of 9p (loss of CDKN2A gene). FISH results over this period consistently detected CRLF2 and IGH gene rearrangements and deletion of 9p21 (loss of CDKN2A gene).

Most recent bone marrow aspirate:

ΔN:™ Flow Cytometry

Analysis/Conclusions: The findings reveal suspicious cells that are present at a level below the lower limit of enumeration for this assay (<0.02%). (See comment.)

Comment: Rare cells are suspicious for abnormal B-lymphoblasts that are present at a level below the lower limit of enumeration and have an immunophenotype like that of the previously detected leukemic blasts (with increased CD34 and decreased CD38). The suspicious cells express CD19, CD22 (slightly increased), CD34 (increased), CD38 (decreased), and CD45.

FACS was performed to isolate the CD3+ T cells and CD19+ B-cells followed by FISH studies.

FISH

Result: 1) CD3+ T cells CRLF2 Xp22.33/Yp11.2 Normal
2) CD19+ B cells CRLF2 Xp22.33/Yp11.2 Abnormal

Interpretation: These findings reveal a low-level CRLF2 rearrangement in the CD19-positive B cell fraction with no evidence of deletions of 9p21 or IGH gene rearrangement.

Conclusion: Patient demonstrates a very low level of B-ALL exhibiting CRLF2 rearrangement with potentially poor prognosis.