Flow Cytometry Immunophenotyping News from ASH 2013
Chapter 1

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The 55th Annual meeting of the American Society of Hematology was held in New Orleans between 7th and 10th December of 2013. More than 3,000 oral and posters abstracts were admitted, and among them, 45 were found after introducing "flow cytometry" and "immunophenotyping" as key words search criteria. European groups leaded the ranking with 26 presentations, followed by the North Americans (n=9), Asian (n=8), and South American (n =2) groups.

The content of these works has been distributed into 2 chapters. In chapter 1, abstracts related to the following sections are included: acute leukemia, minimal residual disease in acute leukemia, myelodysplastic syndrome, and myeloproliferative neoplasms. In chapter 2, the sections included are chronic myeloid leukemia, lymphocyte subpopulations, mature B-cell neoplasms, multiple myeloma and related disorders, paroxysmal nocturnal and platelets and erythrocytes. A list of most used abbreviations is provided at the end of this review.

ACUTE LEUKEMIA

Considering its relevance, not many abstracts have been included within this section, and their content is heterogeneous. **M. Kull *et al.*** presented a gating strategy integrating cells with higher CD45 expression and moderate SSC in order to improve the diagnosis of multicolor FC in a large series of patients diagnosed with NPM1 mutated AML (n=263) (Abstract 2593).

N. Iriyama *et al*. studied the immunophenotype of AML patients with normal karyotype, and identified $CD4^-/CD7^+/CD15^+/CD34^+$ as a particular entity that indicated the presence of the CEBPA mutation, associated with favourable outcome (Abstract 2608).

Finally, **F. Bellos *et al*.** examined the expression of the human equilibrative nucleoside transporter 1 (hENT1) by multiparameter flow cytometry in newly diagnosed AML and MDS. According to their data, lower risk MDS cases and AML with genetic and molecular genetic good risk profile had higher hENT1 expression in several mature and immature cell populations (Abstract 2582).

MINIMAL RESIDUAL DISEASE (MRD) in ACUTE LEUKEMIAS

Most of these abstracts focused on AML. Applicability of MRD in this immunophenotypically heterogeneous disease and the significance of MRD levels after therapy are frequent sources of concern among authors.

F. Lacombe *et al*. presented the results from a French multicentre study designed to evaluate the usefulness of FC for detecting MRD in patients diagnosed with AML (n=307). BM samples were studied after induction and at the end of treatment, with an optional control before the second consolidation. In the end, 274 patients had at least on follow-up sample. Their FC panel

comprised 10 five-colours tubes, but all patients could be assessed for MRD with only 2 tubes. According to their data, the combination of CD45 with CD13, CD33, CD117 and CD34, and additional information provided by CD5 and CD7 represents a quasi-universal panel. Finally, a detailed description of the strategy followed for FC sample analysis, using direct comparison of several samples from the same patient and/or samples from normal BM, was also provided. (Abstract 2613)

Two different groups evaluated the prognostic impact of MRD level on the BM of patients with AML after therapy. **MB. Vidriales *et al*.** analyzed BM samples at CR after induction therapy in 306 non-APL AML patients with different cytogenetic risk subgroups. The Spanish group categorised MRD according to 3 thresholds levels (≥0.1%; ≥0.01-0.1%; and <0.01), and observed that MRD yielded relevant information on favourable (poor prognosis for high MRD levels) and adverse (undetectable MRD overcomes adverse prognosis) cytogenetic groups. They also questioned whether the modality of intensification therapy modified the influence of the level of MRD assessed after induction therapy and concluded that allogeneic transplant is the preferable option for patients with high MRD levels after induction therapy independently of the cytogenetic signature. Finally, this group created a scoring system combining MRD levels and cytogenetics, 5 significantly different AML risk groups could be defined for scores of 0 to 4. These groups had 5-year RFS of 100% (n = 4), 70% (n = 30), 54% (n = 102), 35% (n = 103) and 19% (n = 19), respectively ($p < 0.001$) (Abstract 2576).

Y. Ono *et al*. also studied MRD in non-APL AML, but focused their retrospective study at the end of consolidation therapy. Using 3-color and 6-color strategies, they showed that 70.4% of 81 patients had leukemia associated phenotypes applicable for MRD. Besides, relapse-free-survival was significantly inferior in patients with positive MRD (defined as ≥0.2%). MRD also predicted for early relapses at the end of consolidation (Abstract 3921).

The significance of MRD in AML was also studied in 34 children by the group of **D. Keino *et al*.** Using 4-four-colour FC and a threshold level for MRD-positivity set at 0.1%, they concluded that MRD monitoring might have a prognostic relevance in childhood AML with intermediate risk cytogenetics and negative FLT3-ITD (Abstract 1416).

Finally, **W. Kern *et al.*** made a comparative assessment of MRD by using FC and molecular genetics (MG) in 252 patients with AML (420 BM samples). They evaluated MRD in 4 different time-periods after diagnosis, and in 3 of them (≤60 days after start of therapy, 121-365 days, >1 year), both FC and MG MRD levels had significant correlation with the event free survival. For all four time-periods, MG MRD was significantly related to overall survival (Abstract 54).

The significance of MRD detection in BM aspirate specimens from adult ALL patients (52 B-ALL, 19 T-ALL, 2 T/myeloid leukemia) was investigated by **R. Belizaire *et al*.** using a single tube 6-color FC assay. The 6-marker MRD panel was customized for each patient based on the 18-20-markers studied at diagnosis. Patients were tested after induction or re-induction therapy and serially thereafter. FC MRD showed 86% concordance with the results of morphology. 9% of patients with MRD-negative results relapsed during a median follow-up period of 22 months, and 50% with a MRD-positive result relapsed during a median follow-up period of 15 months ($p=0.001$). Significant differences in the relapse-related and overall mortality between patients with MRD-positive and negative results were also observed (Abstract 1378).

MYELODYSPLASTIC SYNDROME (MDS)

The increasing interest of FC in MDS has been reflected in the number of abstracts dedicated to this disease. Most of them validate its diagnostic use, and many also explore its prognostic significance and its value for monitoring therapy.

W. Kern *et al*. compared FC findings in peripheral blood (PB) from patients with proven MDS (n=96), no MDS (n=32) and MPN, MDS/MPN, or "MDS possible" (n=29). The following 4 criteria were evaluated in PB: presence of myeloid progenitor cells, aberrant expression of ≥1 antigen in myeloid progenitor cells, aberrant expression of ≥2 antigens in granulocytes, and aberrant expression of ≥2 antigens in monocytes. Within the group of patients with proven MDS and non-MDS, 89.5% of the patients who had ≥1 of these criteria had MDS, but MDS was also present in 53.8% of cases without any of these criteria. Strengthening the selection to presence of ≥2 of the criteria, their results confirmed an improvement in specificity and a worsening in sensitivity (Abstract 2774).

E.M.P. Cremers *et al*. analyzed the role of FC in the integrated diagnostic work-up of 316 patients with unexplained cytopenias. In their prospective study, FC reached a sensitivity of 83% and a specificity of 77% for the diagnosis of MDS. The positive predictive value was 78% and the negative predictive value 83%. In the 114 cases without conclusive morphology and where cytogenetics did not add information to the diagnosis, FC identified 11 patients with a typical MDS profile, 28 with dysmyelopoiesis and 69 without any of these signs. Based on their data of FC negative predictive value, the authors postulate that most of these 69 inconclusive cytopenic cases were not MDS. They concluded that FC is instrumental for discriminating clonal and non-clonal causes of cytopenia by excluding underlying MDS when validated standard diagnostic tools such as CM and CCG are not informative (Abstract 1522).

The same Dutch group presented their FC data within the HOVON89 study which evaluates the efficacy of multiple diagnostic tools in patients with low-intermediate-1 risk MDS. The FC analysis was performed according to European LeukemiaNet guidelines, and patients were divided into 3 categories: not likely MDS, signs of dysmyelopoiesis, and fitting MDS. This FC score diagnosed 69/78 patients as probably or likely MDS. FC failed to recognize some patients with only dyserytropoiesis and dysmegakaryopoiesis by morphology not evaluated by the current scoring system. Finally, the authors postulated that patients with unilineage dysplasia by morphology but with multilineage dysplasia by FC, might have a more aggressive disease, pending of confirmation with clinical follow-up. (Abstract 1521)

Patients with low-risk MDS were also the objective of the study of **P. Font *et al*.** Because a morphological BM blast cell <5% has been demonstrated of prognostic value in patients with MDS, these authors explored whether quantification of $CD34^+$myeloid cells using FC might be a reproducible tool among FC observers, and had any correlation with morphology blast count. Six FC observers from different centres analysed, in a blind manner, 47 FC files from BM samples of patients with MDS and <5% blast cells. According to their results, FC turned out a reproducible tool to quantifying $CD34^+$ cells, with a strong agreement among observers (intraclass correlation coefficient of 0.720). It also showed a good concordance on the quantification of $CD34^+$ cells at the critical level of 2% (k=0.587). On the other hand, the lack of a precise correlation between morphology blast cell count and the number of $CD34^+$cells by FC illustrates the importance of considering each value independently (Abstract 2769).

U. Oelschlaegel *et al*. had recently demonstrated a strong association of the MDS del(5q) with a specific 5-parameter FC profile including >2% myeloid progenitors (= 3 points), CD45 MFI

ratio ≤ 7.0 (= 10 points), SSC ratio granulocytes: lymphocytes < 6.0 (= 2 points), ≤ 20% CD71 on granulocytes (= 1.5 points), and female gender (= 1.5 points). In this prospective study on 52 patients with MDS del(5q), they evaluated the use of the FC profile for monitoring response to therapy with drugs like lenalidomide and azacitidine. Before therapy, the 5-FC score was predictable of del(5q) in 92% of cases. After therapy, all cases (n=18) being in cytogenetic CR were also accompanied by the disappearance of the del(5q) FC profile (specificity=100%). However, a FC-score ≥ 15 was found in patients without cytogenetic CR (sensitivity: 65%). The authors conclude that their 5-parameter del(5q) FC profile can serve as a surrogate for the presence of a del(5q) and used as a rapid tool for response monitoring during treatment. They also pointed out a hypothetical role of TP53 mutations in modifying the FC-profile value of some patients (Abstract 2780).

Using FC, **S. Machherndl-Spandl *et al*.** described a significantly lower or even absent expression of major Coxsackie-Adenovirus Receptor on $CD105^+$ erythroid progenitor cells in 20 of 30 patients with MDS (67%), and related BM neoplasms. This expression remained normal in almost all reactive conditions, deficiency states and lymphoid malignancies. The authors propose an abnormal CAR expression as a potential indicator of (clonal) dysplastic erythropoiesis in myeloid malignancies (Abstract 2818).

A. Olsnes Kittang *et al*. investigated the role of myeloid derived suppressor cells (MDSCs) in 24 MDS patients. MDSCs were stained for intracellular TGF-β and IL-10, and were positive for Arginase-1. These cells and Treg numbers were assessed in PB and BM. The authors found increased levels of MDSCs, particularly in high-risk disease, compared to healthy donors. In RAEB patients, numbers of MDSCs were positively correlated with Treg numbers (Abstract 2766)

MYELOPROLIFERATIVE NEOPLASMS

Detection of microparticles using FC is usually part of experimental investigations, but **W.Depei *et al.*** applied microparticle enumeration to the study of a group of 67 patients with MPN with different prothrombotic states. They found a higher level of microparticles in MPN patients compared with healthy controls, and especially in those complicated with thrombosis and splenomegaly, and those with JAK2V617F mutation (Abstract 2368).

CHRONIC MYELOID LEUKEMIA (CML)

FC is not a usual tool for studying patients with CML, but **P. B. van Kooten Niekerk *et al.*** focused their study in these patients and described the $CD34^+$ primitive hematopoietic stem cells subpopulations based on the expression of the human myeloid inhibitory C-type lectin-like receptor (hMICL). They found that, as compared to normal donors, the hMICL+ progenitor subpopulation size varied considerably among CML patients at diagnosis. It also correlated with higher WBC and neutrophil counts, large spleen size, and low hemoglobin levels, so the authors suggest that expansion of this fraction within $CD34^+$cells might reflect a more advanced form of the disease at diagnosis of CML. (Abstract 2704).