Fourth international ELN Workshop on Standardization of Flow Cytometry in MDS
November, 3-5, 2011, Pavia, Italy

Summary

In summary, the working group has agreed that FC adds significantly to MDS characterization, diagnosis and prognosis. It may also be useful in predicting and monitoring disease during treatment with new and standard therapeutic regimens. Repeated FC assessments are strongly recommended not only in cases such as ICUS and IDUS, but also to monitor the natural course of the disease in patients with untreated low and intermediate-1 risk MDS. The same holds true for patients treated with currently available drugs, preferably within clinical trials as conducted by national and international collaborating groups.

Scientific content of and discussion at the meeting

Flow cytometry in MDS as a diagnostic tool

Patients classified by the WHO2001 as having dysplasia in two or three cell lineages (refractory cytopenia with multilineage dysplasia, RCMD) have significantly worse overall survival (OS) and increased risk of leukemic transformation than those with refractory anemia (RA), irrespective of the presence or absence of ring sideroblasts (RS). FC might help to dissect true RA from RCMD by identifying RA with immunophenotypic abnormalities in the myeloid compartment from RA with only erythroid dysplasia.

The new WHO2008 classification identifies three separate entities within the MDS subgroup of refractory cytopenia with unilineage dysplasia (RCUD): refractory anemia (RA), refractory neutropenia (RN) and refractory thrombocytopenia (RT). This classification relies on the lineage displaying more than 10% of dysplasia, and either uni- or bi-cytopenia. It is unclear whether or not RA, RN, RT are distinct MDS subtypes with respect to survival or risk of leukemic transformation. Another newly defined subgroup of MDS is unclassifiable MDS or MDS-U where dysplasia is seen in less than 10% of cells in one or more lineages but associated to cytogenetic abnormalities. The role of FC within RCUD and MDS-U is not yet established but it might allow to identify different disease entities with different prognoses. Moreover, MDS-U patients should be separated from those with cytopenias, who do not meet the criteria of MDS (e.g. with normal karyotype) and have no other

underlying condition that could explain cytopenia. This latter condition is now recognized as idiopathic cytopenia of undetermined significance (ICUS). In contrast to idiopathic dysplasia of undetermined significance (IDUS), patients with ICUS have no major dysplasia but are defined by unexplained cytopenia. IDUS can be considered as a potential pre-phase of MDS. FC could be of value to support the diagnosis IDUS, discriminate between IDUS and MDS and identify IDUS cases that will remain stable rather than progress to acute myeloblastic leukemia (AML) or myeloproliferative neoplasm. Finally, the WHO classification separates MDS with isolated del(5q) as a distinct entity since these patients are characterized by specific morphologic and clinical parameters and have a low risk of evolution to AML. FC may add information to identify 5q- patients with additional immunophenotypic abnormalities who may be at increased risk of evolving to overt AML. This would be important to properly assign these patients to treatment with lenalidomide.

It has been shown in a prospective study that in cytopenic patients with a non-diagnostic BM, FC can effectively aid in distinguishing reactive or secondary chronic cytopenias from myeloid neoplastic diseases. A negative predictive value of 96% by FC could be achieved in patients without significant morphological dysplasia or cytogenetic abnormalities. In addition, FC aberrancies in immature progenitors are highly specific for diagnosing MDS albeit with a lower sensitivity. Recently, a FC test to diagnose low risk MDS patients was designed based on four cardinal parameters i.e the percentage of myeloid progenitors, B cell progenitors, CD45 expression on myeloid progenitors and neutrophil hypogranularity as assessed by sideward light scatter (SSC), which could be confirmed in a multicenter study. Results revealed a sensitivity of 70% and a specificity of 92%.

Flow cytometry in MDS and prognosis

It has been shown that granulocytic and monocytic dyspoiesis in MDS, identified as immunophenotypic abnormalities by FC, correlates with the International Prognostic Scoring System (IPSS), the WHO-adjusted prognostic scoring system (WPSS), transfusion dependency, and time-to-progression to advanced MDS/AML as well as with outcome after hematopoietic stem cell transplantation. Immunophenotypic aberrancies on myeloid progenitors may also have an independent prognostic impact even if the percentage of blasts in the BM is below 5%.

The IPSS represents the benchmark for clinical trials and treatment decision-making in MDS. IPSS is currently under extensive re-evaluation with notably a re-categorization of cytogenetic aberrations. IPSS also does not include yet newly defined prognostic parameters such as co-morbidity scores, serum LDH, BM fibrosis, circulating blasts (normal or aberrant), methylation status, single nucleotide polymorphisms analysis (SNP), mutational status and transfusion requirements. It was shown recently, that cytogenetic abnormalities typically associated with MDS, such as monosomy 7, del(5q) and

complex cytogenetics are correlated with an increased flow cytometric score (FCSS ≥2), whereas chromosomal abnormalities such as trisomy 8, del(20q) and loss of Y, which may also occur in other hematological neoplasms, more frequently display lower FCSS. This confirmed previous data that among patients with lower risk MDS, FC abnormalities were less prominent in patients with trisomy 8 or del(20q). Moreover, the number of FC aberrancies identified in MDS has been reported to be associated with OS, The FCSS extends the prognostic utility of FC assessment especially in RCMD. Although the (revised)-WPSS provides an extended prognostic algorithm compared with the IPSS, FC may further refine this prognostic model.

Evidence for the role of immune dysregulation in MDS pathogenesis is becoming stronger. Indeed, the number and functional status of $CD4^+$ and $CD8^+$ T-cells, NK cells and monocytes are correlated with disease severity. These features also allow differentiating between low risk MDS and aplastic anemia. These markers may add to the FC platform but are not yet included routinely.

Flow cytometry in predicting and monitoring treatment response and disease progression in MDS

The presence of immunophenotypically aberrant myeloid progenitors is instrumental in predicting the response to growth factor treatment. Patients with low serum erythropoietin (Epo) and immunophenotypically normal myeloid progenitors have a high probability (94%) to respond to growth factors. By contrast, patients with aberrant myeloid progenitors and/or high serum Epo levels have a low probability (11%) to respond to treatment. In addition, the degree of phosphorylation of ERK as assessed by FC correlates with response to Epo treatment and OS in low/int-1 risk MDS. (3Disease monitoring by FC may be important especially when other disease parameters such as hematological, molecular and cytogenetic parameters are normal or uninformative about the expected response to therapy. Preliminary studies indicate that MDS-related FC abnormalities in BM cells are no longer detectable or have decreased in responding patients when compared to pre-treatment results. Stable or increased FC aberrancies during treatment may spare patients from long-term treatment with ineffective drugs. This may be of importance in patients treated with hypomethylating agents such as azacitidine who may benefit from an increased OS. Studies are currently ongoing to identify, by intensive FC monitoring, patients who may benefit from prolonged treatment with azacitidine.

Assessment of results and impact of the meeting on the future direction of the field

The results provided by the WP8 of the ELN have major impact on new diagnostic strategies in MDS. This is of particular importance since new drugs are emerging in both lower and higher risk MDS. In addition, the Working Group may provide evidence that FC may add in new prognostic

scoring systems. To this end the Pavia Meeting in 2011 will focus on designing prospective multicenter studies to emphasize diagnostics and prognostics in MDS which might be included in the new WHO classification of hematopoietic neoplasms in [anticipated] 2015.

Final programme

Thursday 3 November 2011

20.00 – 23.00	Dinner in Pavia (Cascina Scova)

Friday 4 November 2011

Aula Goldoniana - Collegio Ghislieri, Pavia

08.30 - 09.10	Registration and coffee
09.10 - 09.20	Welcome and outline of the workshop Bernasconi, Cazzola, Della Porta (Italy)
09.20 - 11.00	Current concepts in diagnostics and prognostics in MDS pathogenesis Chair: Kern (Germany) a) Bone marrow fibrosis: Porwit (Sweden) b) Revised-IPSS and WPPS: Cazzola/Malcovati (Italy) c) Comorbidity assessment: Della Porta (Italy)
11.00 - 11.20	Tea and Coffee
11.20 - 13.00	Current knowledge on flow cytometric evaluation of normal and dysplastic erythropoiesis Chair: Della Porta (Italy) a) An update: Bene (France) b) New antibodies for erythroid dysplasia: Loken (USA) c) Dyserythropoiesis and cytology: Westers (The Netherlands) d) Erytropoiesis and ImageStream: Feuillard (France)
13.00 - 14.30	Lunch
14.30 - 17.00	Plenary working session: evaluation of dyserythropoiesis of 400 samples by the working group: Chair: van de Loosdrecht (The Netherlands)
14.30 – 15.30	a) data presentation: Westers (The Netherlands)
15.30 – 16.00	Tea and Coffee break

16.00 – 16.30 b) pitfalls/technical considerations: Westers (The Netherlands)

16.30 – 17.00 c) Final recommendation: pre-work of document on erythropoiesis

19.00 – 20.00 Cafè al Demetrio – Pavia

20.00 – 23.00 Dinner in Pavia (Locanda Del Carmine)

Saturday 5 November 2011

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09.00 - 10.50 Computed data analysis of flow cytometry

Chair: Orfao (Spain)

a) Data analysis and expression of fluorescence parameters as median/mean: Cullen (United Kingdom)
b) Principles of Infinicyt: Orfao (Spain)
c) An update on the Implementation of Infinicyt in MDS analysis: te Marvelde (The Netherlands)

10.50 - 11.10 Tea and Coffee break

11.10 - 12.30 Review of current scoring systems in MDS

Chair: Loken (USA)

a) diagnostics: Ogata (Japan) and Loken (USA)
b) prognostics: Matarraz (Spain) and Chu (Taiwan)

12.30 - 13.30 Lunch

13.30 - 15.00 Weighing of flow parameters:

Chair : Ireland (United Kingdom)

a) A multivariate analysis of flow parameters in MDS regarding prognostification: towards a risk adapted model: Alhan (The Netherlands)
b) Predictive value of flow in MDS treatment: Burbury (Australia)
c) Proposal of a multicenter analysis: Kern (Germany)

15.00 – 15.30 Conclusions and future work programme for 2012 Workshop
Van de Loosdrecht (The Netherlands)

Final remarks and close of meeting: Della Porta (Italy)