## C11. **EuroFlow panels and strategies for the diagnosis of myelodysplastic syndromes**

Alberto Orfao[1], Sergio Matarraz[1], Vincent H.J van der Velden[2], Juan Flores-Montero[1], Jeroen te Marvelde[2], Quentin Lécrevisse[1], María Belén Vidriales[3], Carlos E. Pedreira[4], Elaine S. Costa[4], Lukas Sedek[5], Tomas Kalina[6], Sebastian Böttcher[7], Ester Mejstrikova[6], Margarida Lima[8], Elizabeth Macintyre[9], Alita J. van der Sluijs-Gelling[10], Tomasz Szczepanski[5], Julia Almeida[1], Jacques JM van Dongen[2].

*[1]Cancer Research Center (IBMCC-CSIC/USAL), Department of Medicine, Cytometry Service and IBSAL, University of Salamanca, Salamanca, Spain;*

*[2], Department of Immunology, Erasmus MC, Rotterdam, The Netherlands;*

*[3], Hematology Service, University Hospital of Salamanca, Salamanca, Spain;*

*[4], Federal University of Rio de Janeiro, Rio de Janeiro, Brasil;*

*[5], Department of Pediatric Hematology and Oncology, Medical University of Silesia, Zabrze, Poland;*

*[6], Department of Hematology/Oncology, Charles University, Prague, Czech Republic;*

*[7], Department of Medicine, University of Schleswig-Holstein, Kiel, Germany;*

*[8], Department of Hematology, Hospital Santo Antonio, Porto, Portugal;*

*[9], Hematology laboratory, Necker-Enfants Malades Hospital, Paris, France;*

*[10], Dutch Childhood Oncology Group, The Hague, The Netherlands.*

In the last two decades, there has been an increased amount of information about the immunophenotypic profiles of normal and myelodysplasia (MDS)-associated hematopoiesis. This includes both detailed knowledge about the immunophenotypic patterns of early committed CD34+ hematopoietic progenitors and precursor cells (HPC) and the features of maturing neutrophils, monocytes, erythroid, basophil, mast cells, dendritic cell, B-cell and T lymphoid precursors, in normal/reactive bone marrow compared to MDS. In order to evaluate different maturational compartments of hematopietic cells, in 2012, the EuroFlow Consortium has proposed a validated 8-color MDS panel of antibodies to be used in combination with standardized sample preparation procedures and new software tools for reproducible analysis of normal vs dysplastic hematopoiesis in the clinical settings. Overall the EuroFlow MDS panel includes up to a maximum of 7 antibody combinations from which the first four are considered essential, while the other three tubes are devoted to obtain complementary information in selected cases.

In detail, all 7 combinations include a backbone of 4 markers which are common to all of them (CD34, CD117, HLADR and CD45) and that are combined with another 4 markers that vary among the different combinations (tube specific characterization markers). The backbone markers repeated in every combination are devoted to the reproducible analysis per tube of the early stages of hematopoiesis in CD34+ and/or CD117+ immature precursors. Thus, such markers already allow for clear discrimination of the early stages of CD34 commitment toward the neutrophil / erythroid / megakaryocytic / T vs mast cell vs B-lymphoid / monocytic / dendritic cell lineages based on the pattern of expression of CD117 and HLADR; in addition, usage of SSC and CD45 expression permits further discrimination between neutrophil, T-lymphoid and erythroid precursors, at the same time it also contributes to the separation between B-lymphoid vs monocytic/dendritic cell precursors at the CD34+ and/or CD117 stages of maturation.

In tube 1, the four variable markers (CD13, CD11b, CD16 and CD10) are specifically devoted to a highly detailed analysis of the neutrophil maturation, although they are also informative for the assessment of other (e.g. monocytic) hematopoietic cell compartments. Therefore, based on this 8-color combination, neutrophil committed precursors from normal bone marrow are known to sequentially loose expression of CD34, HLADR, CD117 and CD13, followed by acquisition of CD11b, CD13, CD16 and CD10 expression. Similarly, tube 2 is devoted to the specific dissection of the monocytic pathway by combining the CD64, CD35, CD14 and CD300e markers, with the above listed four backbone antigens. CD34+ monocytic precursors can be easily identified by their early acquisition of CD64, followed by loss of CD34 and CD117 and sequential acquisition of CD14, CD35 and finally, CD300e. The third tube in the MDS EuroFlow panel aims at detailed characterization of the erythroid maturation from the earliest CD34+ CD117+, HLADR+ precursors to the more mature nucleated red cells in the bone marrow. Therefore, erythroid maturation is first confirmed by coexpression of CD36 and CD105 on CD34+ precursors that progressively loose expression of CD34, CD33, HLADR and finally CD117, at the same time they show increasing amounts of CD36, CD105 and CD71; subsequently, erythroid precursors loose CD105, and downregulate at the very late stages also CD36 and CD71. With tube 4, assessment of the lymphoid maturation in the bone marrow is achieved through the addition of NuTDT, CD19 and CD7 to the CD34, CD45, CD117 and HLADR backbone markers; in this tube CD56 is used mostly for the assessment of NK-cells and aberrant marker expressions on hematopoietic precursors, monocytic and neutrophil lineage cells.



The remaining three supplementary tubes allow identification of minor myeloid cell lineages (e.g. tubes 6 and 7 permit the identification of basophils, plasmacytoid dendritic cells and megakaryocytic cells and their precursors) whereas tube 5 is devoted to the identification of aberrant phenotypes and immature CD34+/CD38- precursors.

Of note, preliminary data suggests that usage of the EuroFlow MDS antibody panel in combination with the newly developed maturation tools implemented in the INFINICYT software (Cytognos, SL, Salamanca, Spain), allow for sensitive detection of aberrant and altered immunophenotypes among the bone marrow maturing neutrophil monocytic and erythroid cells from MDS patients, with a high accuracy. In addition, it has also proven to be highly efficient for the diagnostic screening of MDS-associated with other lymphoid malignancies e.g. in patients with multiple myeloma. At the same time, usage of the maturation software tools in combination within reference EuroFlow data bases, provides a reproducible basis for the identification of such alterations in suspected patients, provided that highly comparable and reproducible sample preparation procedures and antibody panels had been used.

Altogether, this will most probably facilitate extended usage and application of multiparameter flow cytometry immunophenotyping in routine diagnostic practice for the diagnosis, classification and monitoring of MDS.

**SELECTED REFERENCES:**

1.- KALINA T, FLORES-MONTERO J, VAN DER VELDEN VHJ, MARTIN-AYUSO M, BÖTTCHER S, RITGEN M, ALMEIDA J, LHERMITTE L, ASNAFI V, MENDONÇA A, DE TUTE R, CULLEN M, SEDEK L, VIDRIALES MB, PEREZ JJ, MEJSTRIKOVA E, HRUSAK O, SZCZEPANSKI T, VAN DONGEN JJM, ORFAO A: EuroFlow standardization of flow cytometry instrument settings and immunophenotyping protocols. Leukemia, 26: 1986-2010, 2012.

2.- LOKEN MR, VAN DE LOOSDRECHT A, OGATA K, ORFAO A, WELLS DA: Flow cytometry in myelodysplastic syndromes: report from a working conference. Leukemia Research, 32: 5-17, 2008.

3.- MATARRAZ S, PAIVA B, DIEZ-CAMPELO M, BARRENA S, JARA-ACEVEDO M, GUTIERREZ L, SAYAGUES JM, SANCHEZ ML, BARCENA P, GARRASTAZUL M, BERRUEZO MJ, DURAN J, CERVERO C, GARCIA-ERCE J, FLORENSA L, MENDEZ G, GUTIERREZ O, DEL CAÑIZO C, SAN MIGUEL JF, ORFAO A: Immunophenotypic alterations of bone marrow myeloid cell compartments in multiple myeloma patients predict for myelodysplasia-associated cytogenetic alterations. Leukemia, 2014 (in press).

4.- PORWIT A, VAN DE LOOSDRECHT A, BETTELHEIM P, BURBURY K, CREMERS E, DELLA PORTA M, EIDENSCHINK-BRODERSEN L, IRELAND R, JOHANSSON U, MATARRAZ S, OGATA K, ORFAO A, PREIJERS F, PSARRA K, SUBIRA D, VALENT P, VAN DER VELDEN V, WELLS D, WESTERS T, KERN W, BENE MC. Revisiting guidelines for integration of flow cytometry results in the WHO classification of Myelodysplastic Syndromes - proposal from the International/European Leukemia Net Working Group for Flow Cytometry in MDS (IMDSFlow). Leukemia, 2014 (in press).

5.- VAN DONGEN JJM, LHERMITTE L, BÖTTCHER S, ALMEIDA J, VAN DER VELDEN VHJ, FLORES-MONTERO J, RAWSTRON A, ASNAFI V, LECREVISSE Q, LUCIO P, MEJSTRIKOVA E, SZCZEPANSKI T, KALINA T, DE TUTE R, BRÜGGEMANN M, SEDEK L, CULLEN M, LANGERAK AW, MENDONÇA A, MACINTYRE E, MARTIN-AYUSO M, HRUSAK O, VIDRIALES MB, ORFAO A. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia, 26: 1908-1975, 2012.

6.- VAN DONGEN JJM, ORFAO A: EuroFlow: resetting leukemia and lymphoma immunophenotyping. Basis for companion diagnostics in personalized medicine. Leukemia, 26: 1899-1907, 2012.

7.- VAN LOORDECHT A, ALHAN C, BENE MC, DELLA PORTA MG, DRAGER AM, FEUILLARD J, FONT P, GERMING U, HAASE D, HOMBURG CH, IRELAND R, JANSEN JH, KERN W, MALCOVATI L, TE MARVELDE JG, MUFTI GJ, OGATA K, ORFAO A, OSSENKOPPELE GJ, PORWIT A, PREIJERS FW, RICHARDS SJ, SCHUURHUIS GJ, SUBIRA D, VALENT P, VAN DER VELDEN VH, VYAS O WESTRA AH, DE WITTE TM, WELLS DA, LOKEN MR: Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNET working conference on flow cytometry in myelodysplastic syndromes. Haematologica, 94: 1124-1134, 2009.

8.- WESTERS T, IRELAND R, KERN W, CANAN A, BALLEISEN JS, BETHELHEIM P, BURBURY K, CULLEN M, CUTLER J, DELLA PORTA M, DRAGER A, FEUILLARD J, FONT P, GERMING U, HAASE D, JOHANSSON U, KORDASTI S, LOKEN M, MALCOVATI L, TE MARVELDE J, MATARRAZ S, MILNE T, MOSHAVER B, MUFTI G, OGATA K, ORFAO A, PORWIT A, PSARRA K, RICHARDS S, SUBIRA D, TINDELL V, VALLESPI T, VALENT P, VAN DER VELDEN V, DE WITTE T, WELLS D, ZETTL F, BENE MC: Standardization of flow cytometry in myelodysplastic syndromes: a report from an International Consortium and the European LeukemiaNet Working Group. Leukemia, 26: 1730-1741, 2012.