cytognos
Flow Cytometry Solutions

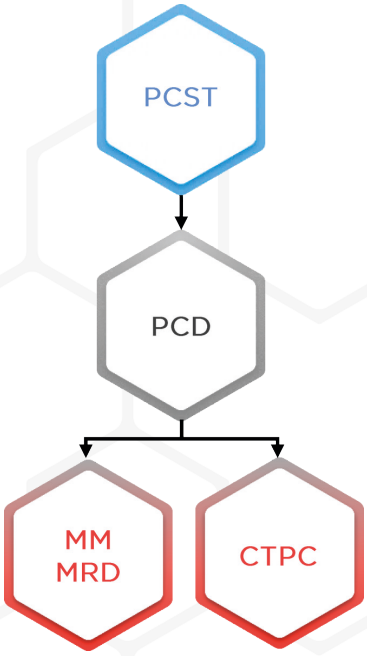
MINIMAL RESIDUAL DISEASE
MM MRD

MULTIPLE MYELOMA

Plasma cell disorders are a heterogeneous group of clinical disorders characterized by the expansion of clonal plasma cells in the bone marrow. The range of plasma cells disorders goes from Monoclonal Gammopathy of Undetermined Significance (MGUS) to symptomatic diseases such as Multiple Myeloma (MM) [1].

An important effort is being made to improve the efficacy of therapies to obtain an increased rate of remission and reduction of the number of pathological cells. Part of the success on this task depends on the sensitivity and specificity of the methodologies employed in diagnosis and follow-up of the patients [2].

IMMUNOPHENOTYPING IN MULTIPLE MYELOMA WORKFLOW



Adapted from van Dongen JJ, Leukemia. 2012 Sep; 26(9):1908-75

Flow cytometry has proved to be an important tool in the diagnosis and classification of MM and other plasma cell disorders throughout the years [1]. It has been demonstrated that abnormal plasma cells often show different phenotypes compared to their normal counterparts, namely the aberrant expression of CD56 in most patients or decreased levels of other molecules such as CD38, CD27, CD45 and CD138. These findings, together with other phenotypical changes, provide an aberrant plasma cell signature that allows for a specific and sensitive detection of the abnormal cells, crucial for an early diagnosis and a reliable follow-up [3].

In recent years, the introduction of new drugs has led to the improved survival of patients with MM [4] The treatment response of these patients has been so far evaluated by techniques that were patient specific and the Minimal Residual Disease (MRD) criteria was not well established since most patient relapsed [2, 4]. The major drawback of flow-MRD was the lack of standardization [3].

THE EUROFLOW™ MULTIPLE MYELOMA MRD PANEL

PATENTED!

BV™421	BV™510	FITC	PE	PerCP-Cyanine5.5	PE-Cyanine7	APC	APC-C750™
CD138	CD27	CD38	CD56	CD45	CD19	CD117	CD81
CD138	CD27	CD38	CD56	CD45	CD19	CyIgKappa	CyIgLambda

EuroFlow™ has designed and validated a flow cytometry based method for MRD evaluation in MM. The developed panel is composed of two separate antibody combinations orientated towards the identification of immunophenotypically aberrant clonal plasma cells in bone marrow (BM) samples. The use of two tubes is an important quality control of the process since the test is performed in two replicates of the sample and also to confirm the clonality of suspected cells. An increased sensitivity due to the high number of cells analyzed is achieved. A sensitivity of at least of 10^{-5} has shown to be clinically meaningfull [2, 5, 6].

There are treatments that specifically target surface or intracellular molecules of abnormal plasma cells. These treatments may block the binding of fluorochrome-conjugated anti-CD38 monoclonal antibodies in flow cytometry studies. Cytognos developed an anti-CD38 antibody which recognizes different epitopes of the antigen and allows for the identification of plasma cells even in the presence of therapeutic anti-CD38 antibodies [2, 6].

The combined use of CD38 and CD138 is currently recommended for the identification of plasma cells in MM workflow and for this reason both have been included in the panel [3, 6]. CD38 has shown to be valuable for the identification of both normal and abnormal plasma cells given its expression pattern significantly different from that of CD38+ precursor cells [2, 3, 6]. CD138 is very specific for plasma cells and, although it presents some downregulation on aged samples, it results useful when combined with CD38, CD45 and light scatter properties [3, 6].

Every marker selected has shown to be relevant for the detection of MRD at a very high sensitivity levels contributing for the separation between normal and abnormal immunophenotypes: CD19 (97%), CD45 (89%), CD56 (86%), CD81 (86%), CyIgλ (73%), CD27 (71%), CD117 (60%) and CyIgκ (56%) [2].

STANDARDIZED OPERATING PROCEDURES FOR MRD EVALUATION

Flow cytometry immunophenotyping results are highly dependent on the sample processing protocols employed. For this reason, EuroFlow™ developed standardized protocols for each panel to assure full technical standardization in 3-laser based cytometers and in the specific case of the MM MRD to detect rare events with high sensitivity [2, 6]. The goal of EuroFlow™ was to reach a sensitivity comparable to real-time quantitative polymerase chain reaction (RQ-PCR)-based MRD analysis and, to achieve that, a minimum of 10 million total cells must be analyzed [2].

During early phases of treatment, the cellularity of BM samples are frequently low and using the standard methodology requiring the direct staining of 100 µl of whole BM will not allow the acquisition of the millions of cells needed. EuroFlow™ developed a new erythrocyte BulkLysis™ procedure to lyse sufficiently large volumes of BM, and resuspend the resulting leukocytes in a small volume of washing buffer suitable for staining. This new protocol allows for the staining of 10 million cells in 100 µL of cell suspension without compromising the data quality [6].

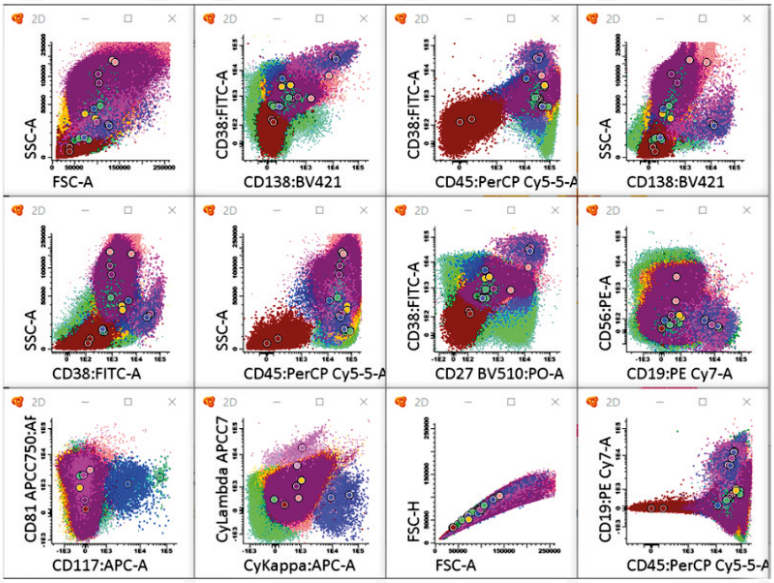
The corresponding Standard Operating Procedures (SOPs) may be found at www.euroflow.org.

INFINICYT™ DATA ANALYSIS AND REFERENCE DATABASES

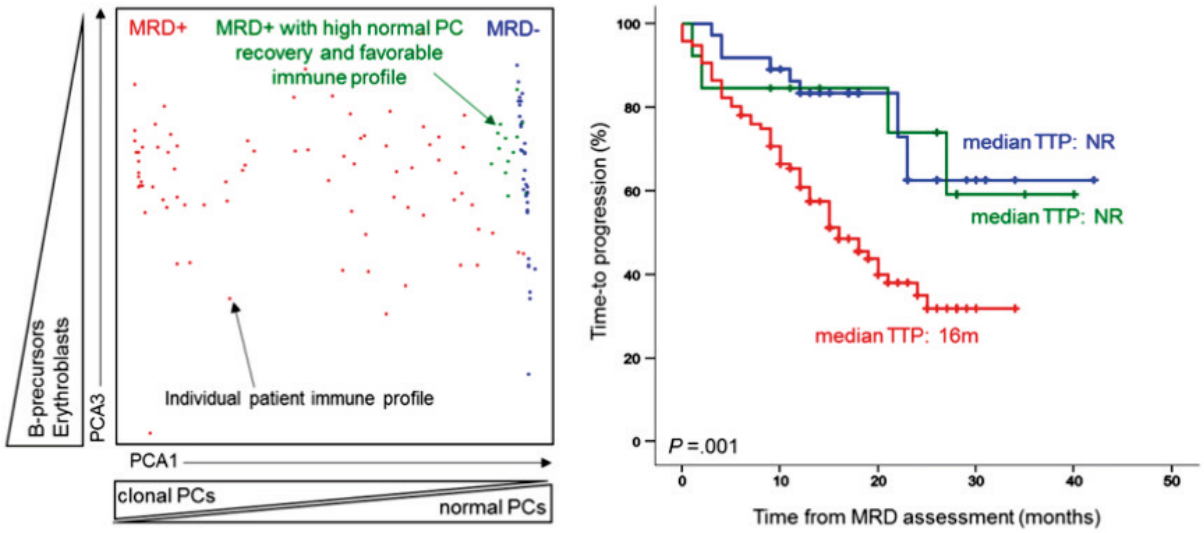
The number of immunophenotypic markers that can be evaluated in 8-color assays and the high number of cells interrogated using the MM MRD methodology increases the complexity of data analysis. EuroFlow™ developed and validated a database containing representative flow cytometry data sets from normal healthy BM samples processed in different standardized centers. The database (available through Infinicyt™) when used with files which follow SOPs allows for an automated analysis of the complete BM sample [2].

The MM MRD database is designed to be used with Infinicyt™ Automated Gating and Identification (AG&I) tool in order to provide a complete analysis of the sample. Normal populations are identified comparing them with the reference database while events which differ from normal need to be confirmed by the user.

Evaluating the complete immune profile of the sample, instead of simply looking for abnormal plasma cells, has shown to be prognostically relevant, allowing for the identification of patients with poor survival. Moreover, the flow cytometry based MRD negativity has shown to be associated with a longer time of progression independently from the risk level established by other techniques (e.g. FISH) [5].



Studies demonstrate that MRD-negative status surpasses the prognostic value of complete remission across the disease spectrum regardless of the type of treatment or patient risk group [2]. MRD negativity should be considered as one of the most relevant assessments as long as the method is performed with a high level of sensitivity and using standardized protocols as proposed by the EuroFlow™ group [5].



Images provided by Dr. Paiva (CIMA LAB diagnostics, Spain)

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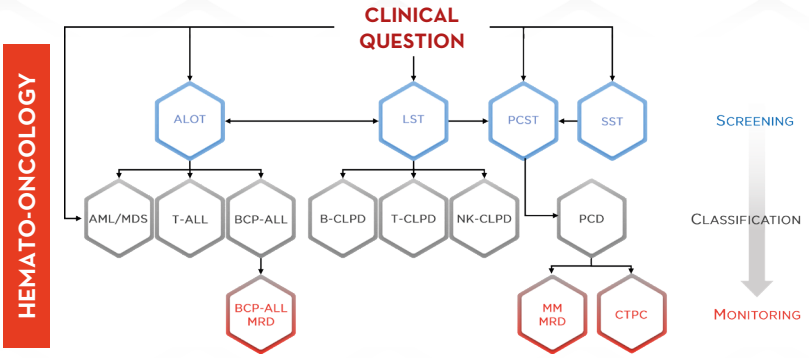
2. Flores-Montero J, et al. Next Generation Flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. Leukemia. 2017 Oct;31(10):2094-2103.
3. Flores-Montero J, et al. Immunophenotype of normal vs. myeloma plasma cells: Toward antibody panel specifications for MRD detection in multiple myeloma. Cytometry part B Clinical Cytometry. 2016 Jan;90(1):61-72.
4. Kumar S, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncology. 2016 Aug;17(8):328-346.
5. Paiva B, et al. Minimal residual disease monitoring and immune profiling in multiple myeloma in elderly patients. Blood. 2016 Jun;127(25):3165-3174.
6. EuroFlow Consortium website: www.euroflow.org.

Product	Reference	Regulatory Status	Format	Size
MM MRD kit	CYT-MM-MRD8	CE-IVD	Lyophilized	20 test
CD38 multi-epitope-FITC	CYT-38F2	CE-IVD	Liquid	50 test



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