ImmunoTools *special* Award 2018

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Immune profiling of patients with Multiple Myeloma (MM) after first-line treatment: Correlations with minimal residual disease (MRD)

Multiple Myeloma (MM) is the second most common hematological malignancy, which is characterized by the uncontrolled proliferation of clonal plasma cells (PCs) in the bone marrow (BM) and excess production of monoclonal protein in the serum and/or the urine. Novel therapies including proteasome inhibitors (PIs), immunomodulatory drugs (IMiDs) or monoclonal antibodies have significantly increased the rates of complete remission (CR; the primary goal of many treatments) using conventional criteria (eg. negative immunofixation in serum and urine) and have improved the clinical outcome of patients. However, MM remains an incurable disease with subsequent relapses and refractoriness. Therefore, the identification of biomarkers that are capable to predict disease progression and relapse is of utmost importance in the clinical management of MM patients with misleading CR.

The assessment of minimal residual disease (MRD; i.e., the presence of very few clonal PCs remaining in patients' BM after treatment) has emerged as an excellent biomarker during the course of the disease, as MRD positivity has been correlated with shorter progression-free survival (PFS) and inferior overall survival. Of note, the quantification of MRD tumor load seems to be of great importance, since a subgroup analysis of MRD positive (MRD^+) patients showed that higher numbers of residual aberrant PCs (on a logarithmic scale) could independently predict a more adverse clinical course. These observations necessitate the application of highly sensitive techniques that will allow for the detection of very small numbers of clonal populations within the BM of treated MM patients. Nevertheless, the specific characteristics of MRD^+ vs. MRD negative (MRD^-) patients and, most importantly, the biology underlying disease progression is largely unexplored.

In our lab, we have efficiently established a highly sensitive and standardized multicolor flow-cytometry approach for the evaluation of MRD at the level of 10-6, according to the EuroFlow guidelines. The multiparametric nature of our technique allows not only for the discrimination of rare clonal PCs, but also for the profiling of the major BM subsets (e.g. naïve and memory B cells, T cells, erythroblasts, myeloid progenitors, monocytes, neutrophils) within the BM niche. Undoubtedly the BM niche is a very dynamic environment which, on one hand, provides a supportive milieu for tumor PCs, but at the same time, hosts a large variety of immune subsets seeking to control tumor PC propagation. Based on our initial results showing significant

differences in the relevant distribution of specific BM subsets (i.e. erythroblasts, monocytes) between MRD^- and MRD^+ patients, we hypothesize that immune profiling of treated MM patients will also reveal a differential immune status associated with the presence and the level of MRD.
Immunotools antibodies will contribute to our effort for the in-depth immune profiling of the BM niche in treated MM patients, where tumor cells interact with both effector and immunesuppressive cells. We will focus on the relative distribution of cells with cytotoxic activity (i.e., $CD8^+$ T cells, NK and NKT cells) and those with immune-suppressive functions (e.g., Tregs) in the BM of MRD^+ and MRD^- MM patients, and we will also perform a detailed subset analysis of these populations to gain better insight of their functional status. Accordingly, the immune status of MM patients will be further characterized by analyzing indicative stimulatory (e.g IFN-gamma) and suppressive cytokines (e.g. IL-10), using the appropriate ELISA sets provided by Immunotools.
We believe that this approach will highlight important aspects of the tumor immunoediting processes within the BM and may unveil mechanisms linked to MRD biology and the selective survival of tumor cells variants that escape immune surveillance leading eventually to disease relapse.

Identification and characterization of CD8 cytotoxic cells: CD4, CD8, CD9, CD11a CD44, CD62L

Identification and characterization of NK cells: CD11b, CD16, CD56, CD27, CD57

Identification and characterization of Tregs: CD4, CD25, CD45RA, CD45RO, CD127, HLA-DR

ImmunoTools *special* AWARD for **Ioannis Kostopoulos** includes 25 reagents

APC – conjugated anti-human CD11a, CD25, CD27, CD44

FITC - conjugated anti-human CD4, CD16, CD57, CD127

PE - conjugated anti-human CD9, CD45RO, CD56, CD62L

PerCP - conjugated anti-human CD4, CD8, CD11b, CD45RA, HLA-DR

human ELISA-set (for one 96 plate): human IFN-gamma, human IL-10

DETAILS more AWARDS