ImmunoTools *special* Award 2017

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Blockade of immune checkpoints in Multiple Myeloma

Co-stimulating receptors CD80/CD86 on the surface of Antigen Presenting Cells (APC) bind to CD28 delivering a positive signal to complete T cell activation. Conversely, CD80/CD86 can also bind cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) on T cells triggering an inhibitory response. After CD28 and CTLA-4 discovery, additional co-stimulatory molecules (CD137, OX40, CD226, GITR, etc.) and co-inhibitory molecules (PD1, LAG-3, TIGIT, TIM-3, etc.) have been identified and collectively named immune checkpoints. A better understanding of these immune checkpoints will be crucial for the development of new therapies for immune-mediated diseases including autoimmune diseases, graft-versus-host diseases and cancer.

Recent advances in therapies targeting immune checkpoints have opened up the prospect of promising developments in tumour immunology. The first cancer immunotherapy based on blockade of immune checkpoints was a blocking anti-CTLA-4 mAb (Ipilimumab) FDA-approved to treat metastatic melanoma in 2011. Soon after, effective immunotherapies have been developed targeting the co-inhibitory molecule PD-1 and its ligand PD-L1. These new immunotherapies against cancer became the main breakthrough of 2013 according to the Science journal. Thus, this emerging topic of research offers extraordinary opportunities to improve therapeutic approaches in cancer.

It is now crucial to elucidate the specific immune checkpoints that are involved depending on the cancer type and the stage of disease. Our research focuses on identifying relevant immune checkpoints in active multiple myeloma (MM) and the pre-malignant condition monoclonal gammopathy of undetermined significance (MGUS) that usually precedes MM. MM is a clonal B-cell malignancy that is characterized by the neoplastic proliferation of a plasma cell (PC) clone that produces a monoclonal immunoglobulin which usually results in organ or tissue impairment (hypercalcemia, renal failure, anaemia, bone lesions or extramedullary plasmacytomas). In Western countries, MM accounts approximately for 13% of hematologic cancers and its frequency is likely to increase in the near future as the population ages. MM remains incurable although the introduction of autologous stem-cell transplantation (ASCT) and the availability of new agents such as

thalidomide, lenalidomide and bortezomib have recently increased the median survival to 6.1 years.

In order to identify immune checkpoints that might be controlling the proliferation of abnormal plasma cells in patients with MGUS and their progression to malignant MM, we will compare the expression of immune checkpoints in bone marrow samples from patients with MGUS, active MM, and MM in remission. Immunophenotyping by flow cytometry will be crucial to discover the specific cell subset/s involved in immune regulation in bone marrow from MGUS and MM patients. Plasma cells will be evaluated using a 7 color panel: CD45, CD38, CD138, molecule of interest and aberrant expression of CD19, CD56 and CD117. Furthermore, we will use a second panel to assess immune checkpoint expression such as LAG-3 on immune cells (NK, $CD4^+$ and $CD8^+$ T cells). We will also track presence of Tregs (CD25, CD127, FoxP3). Conjugated antibodies from ImmunoTools will be very useful to determine the expression of immune checkpoints on the immune cell subsets and their intracellular cytokine production. Samples will be analyzed on a flow cytometer following procedures standardized by the EuroFlow Consortium. Gene silencing of molecule of interest by lentiviral transduction and functional assays in the presence of recombinant cytokines from ImmunoTools will also provide evidence of its functional role on PCs. Supernatants will be analyzed by ELISA. Reagents from ImmunoTools will be very useful to better understand the role of immune checkpoints in multiple myeloma.

ImmunoTools *special* AWARD for **Ester Lozano** includes 25 reagents

FITC - conjugated anti-human IL-6, CD69, Annexin V

PE - conjugated anti-human IFN-gamma, IL-8, TNFa

human ELISA-set (for one 96 plate), human IFN-gamma, human IL-4

recombinant human cytokines: rh IL-1 beta, rh IL-4, rh IL-6, rh IL-7, rh IL-9, rh IL-12, rh IL-15, rh IGF-I, rh SDF-1α, rh SDF-1β, rh TNFα

DETAILS more AWARDS