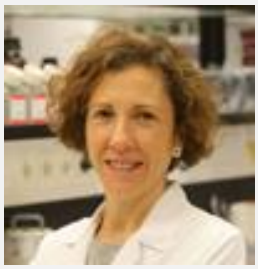
ImmunoTools *special* Award 2014



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Identification of Biomarkers to Select the Repertoire of Tumor-specific T Cells with High Self-renewal Properties. *Implication in Adoptive Immunotherapy of Cancer*

Tumor infiltrating T lymphocytes (TILs) are a type of cell found in tumors that are implicated in killing tumor cells. These cells can be isolated from patients' tumors and grown to very large numbers in the lab. After TIL expansion, patients are infused with their own TILs. This treatment, known as adoptive cell transfer (ACT) of TILs, has been shown to be highly successful at eradicating large refractory tumors in greater than 50% of treated melanoma patients and there is a great interest to apply the TIL-ACT therapy to other common metastatic malignancies, such as metastatic colorectal cancer. However, the implementation of this technology requires the development of more efficient methods to generate TILs with high anti-tumor activity. TILs are a heterogeneous population composed of tumor-specific T cells but also of non-tumor-reactive T cells. A factor that limits the use of TIL-ACT is the low percentage of tumor-specific T cells within the final TIL product infused to the patients. This happens because most of tumor-specific T cells exhibit low self-renewal capacity. During the expansion phase non-tumor-reactive T cells present in the TILs grew more efficiently outnumbering tumor-specific T cells and leading to TIL products with low tumor-reactivity. Recently, a set of biomarkers associated to activation/antigen (Ag) recognition and stemness of T cells have been identified that allow recognition of Ag-specific T cells with high self-renewal properties.

The aim of our study is to test the potential of these biomarkers to select the repertoire of tumor-specific T cells with stem-like properties. First, we will phenotype (by multicolor flow cytometry) TILs from human liver metastatic colorectal cancer patients. ImmunoTools' mAbs, such as FITC-conjugated anti-human CD4/CD16/CD27/CD45 /CD45RA/CD95/HLA-DR mAbs; PE-conjugated anti-human CD27/CD25/CD38 /CD45/CD56/CD57/CD62L/ CD69/CD95 mAbs; PerCP-conjugated anti-human CD8/CD45RA and APC-conjugated anti-human CD3/CD62L mAbs, will be very useful for this phenotyping. Then, cells will be

isolated by flow cytometry-based sorting on the basis of the co-expression of a marker associated to activation/Ag recognition and a marker associated to stemness. Isolated cells will be expanded *in vitro* and further phenotypically characterized. Immunotools' cytokines (rhIL-7 and rhIL-15) are very valuable reagents for this *in vitro* expansion. To assess tumor-specificity, expanded cells will be co-cultured with autologous tumor cells and subsequently analyzed by flow cytometry for the expression of intracellular cytokines using ImmunoTools' PE-conjugated mAbs against human IFNgamma and TNFalpha and mouse IgG1-PE, as isotype control.

This research project, in which flow cytometry and cell expansion is vital, could develop a potent methodology to isolate the repertoire of tumor-specific stem cell-like T cells. This technology, easily transferable to GMP facilities, would have great implications to implement the use of TIL-ACT in the treatment of metastatic malignancies.

ImmunoTools *special* AWARD for **Sandra Hervas-Stubbs**

includes 25 reagents

FITC - conjugated anti-human CD4, CD16, CD27, CD45, CD45RA, CD95, HLA-DR,

PE - conjugated anti-human CD27, CD25, CD38, CD45, CD56, CD57, CD62L, CD69, CD95, IFNgamma, TNFalpha, mouse IgG1 isotype control.

PerCP - conjugated anti-human CD8, CD45RA,

APC - conjugated anti-human CD3, CD62L,

recombinant human cytokines:, rhIL-7, rhIL-15 DETAILS more AWARDS