ImmunoTools *special* Award 2018

João Calmeiro, PhD-student

Supervisor: Dr. Teresa Cruz Rosete and Dr. Bruno Neves

Center for Neuroscience and Cell Biology (CNC UC), Faculty of Pharmacy, University of Coimbra, Polo III, 3000-548 Coimbra, Portugal

Potentiation of Dendritic Cell vaccines for next-generation cancer treatment

Dendritic cells (DCs) are highly specialized professional antigen-presenting cells characterized by their superior capacity for acquiring and processing antigens and for its subsequent presentation to naïve T cells, being responsible for the orchestration of the adaptive immune responses. Given the protective role of the immune system in eliminating tumors, DCs application in immunotherapy have been scrutinized and settled as highly desirable and full of translational and clinical potential *[1].* Since the 1990's, DCs have been used in more than 300 clinical trials as cellular antitumor vaccines. These vaccines are based on the unparalleled capacity of DCs to initiate effective anti-tumor immune responses and empowering the suppression of tumor growth. The main approach exploring DCs in oncologic treatments is vaccines composed of *ex vivo* generated DCs matured and loaded with tumor antigens *[2,3].*

Notwithstanding the good safety profile of DC immunotherapy, the rate of success in inducing clear therapeutic outcomes is variable. Objective tumor responses are usually above 15% and promising vaccines in early phase studies often fail to present clinical benefits in pivotal phase III trials *[4]*. In parallel with the growing knowledge of cellular and cancer biology, DC-based approaches must be optimized given that most of the protocols were already established twenty years ago. Indeed, classical monocyte-derived DCs used in nearly 90% of clinical trials do not possess the ideal functional and phenotypical characteristics required for the induction of a robust anti-tumor immune response. Therefore, it is necessary the development of new technologies and strategies focusing on DC-based vaccines that will boost all its potential, improving cancer vaccines efficacy and harnessing future and innovative cancer therapeutics.

Since there are evidences that the composition of the commercially GMP culture mediums available can influence DCs characteristics and functions, one of the first steps to be optimized in DC vaccine protocols is the type of medium used when monocytes are being differentiate in DCs. Therefore, our group are invested in clarifying which one of the commercially available GMP culture mediums is the best to use in the context of DC immunotherapy against cancer.

With the ImmunoTools reagents, we will be able to induce the differentiation of monocytes into dendritic cells (rh GM-CSF and rh IL-4), as well as verifying the efficacy of this process by the loss of CD14 expression and the augment of CD11c expression using specific fluorescently-labelled mAbs for flow cytometry. The ImmunoTools reagents would also allow us to evaluate the state of maturation of DCs when using standards cocktails, by the analysis of specific molecules (e.g. CD80, CD86, HLA-DR). Furthermore, we can also verify DCs capacity to activate T cells in mixed lymphocyte reactions, analyzing T cell markers (CD3, CD4, CD8, CD25) by flow cytometry, as well as their prodution of IFN-gamma. In order to control the survival and proliferation of T cells, a low amount of rh IL-2 will be added in the mixed culture. Lastly, all the isotype controls from ImmunoTools will allow us to correctly analyze these data by flow cytometry.

[1] R.M. Steinman, Decisions About Dendritic Cells: Past, Present, and Future., Annu. Rev. Immunol. 30 (2011) 1–22. doi:10.1146/annurev-immunol-100311-102839.

[2] J. Constantino, C. Gomes, A. Falcão, M.T. Cruz, B.M. Neves, Antitumor dendritic cell–based vaccines: lessons from 20 years of clinical trials and future perspectives, Transl. Res. 168 (2016) 74–95. doi:10.1016/j.trsl.2015.07.008.

[3] K. Palucka, J. Banchereau, Dendritic-cell-based therapeutic cancer vaccines., Immunity. 39 (2013) 38–48. doi:10.1016/j.immuni.2013.07.004.

[4] S. Anguille, E.L. Smits, E. Lion, V.F. van Tendeloo, Z.N. Berneman, Clinical use of dendritic cells for cancer therapy, Lancet Oncol. 15 (2014) e257–e267. doi:10.1016/S1470-2045(13)70585-0.

ImmunoTools *special* AWARD for **João Calmeiro** includes 24 reagents

FITC - conjugated anti-human CD4, CD11c, CD14, CD80, Control-IgG1, Annexin V

PE - conjugated anti-human CD8, CD25, IFN-gamma, TNFa, Control-IgG1

PerCP - conjugated anti-human CD3, Control-IgG1

APC - conjugated anti-human CD14, CD45, CD54, CD86, HLA-DR, Control-IgG1, Annexin V

recombinant human cytokines**:** rh GM-CSF, rh IL-2, rh IL-4, rh IL-15