ImmunoTools *special* Award 2023

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Development of a Dendritic Cell-based vaccine to halt triple-negative breast cancer

TNBC is characterised by the absence of the progesterone receptor (PR), oestrogen receptor (ER), and human epidermal growth factor receptor 2 (HER2). TNBC is responsible for 15%–20% of new breast cancer diagnoses worldwide. Some TNBC cases arise from inherited germline mutations, with BRCA germline mutations being the most common ones. Compared to other breast cancer subtypes, TNBC is the most aggressive. Indeed, it is estimated that the mortality rate within five years post-diagnosis is approximately 70%. In addition, TNBC patients may develop metastasis to multiple organs, such as the liver, bones, and central nervous system. Central nervous system metastasis develops in approximately 46% of patients with TNBC. As TNBC does not display specific targets, chemotherapy is the main therapeutic approach. Nevertheless, these patients still have an increased risk of recurrence and significantly reduced chance of survival. Recently, immunotherapy has been emerging because of the high immunoreactivity present in a large percentage of TNBC cases, including anti-tumour dendritic cell (DC) vaccines.

DCs, widely known as professional antigen-presenting cells, represent the link between innate and adaptive immunity, and are critical for initiating and orchestrating the immune response by capturing, processing, and presenting antigens to naïve T cells in the lymph nodes. This is one of the key features of DCs as a promising immunotherapeutic tool. In addition to direct antigen presentation, DCs display the capacity to migrate between non-lymphoid and lymphoid tissues, controlling chemokine and cytokine gradients to moderate inflammation and lymphocyte homing, and, of utmost importance, cross-present antigens to $CD8^+$ T cells, also known as cytotoxic lymphocytes (CTLs). All these aspects are crucial for systemic and long-lasting antitumour effects. Owing to this immunotherapeutic potential, *ex vivo* DCs

have been extensively manipulated and studied in several preclinical and clinical studies as anti-tumour agents. Despite their safety and immunogenicity, their clinical response has not been as anticipated, which puts the challenges of treating cancer back into focus after their effective installation. Then, “repurposing” DCs and using them as preventive agents is a possibility for the success of this immunotherapeutic tool.

Taken together, one plausible option to fight TNBC is to act in a prophylactic manner by proactively priming the immune system with DCs loaded with tumour antigens. Then, we advocate the formulation of a prophylactic DC-based vaccine designed to forestall or postpone the onset of TNBC.

To this end, we will perform *in vivo* experiments using an orthotopic mouse model. First, we will isolate cells from the femur and/or tibia of BALB/c mice and differentiate them into DCs using the cytokines rm GM-CSF or rm Flt3L/CD13 from ImmunoTools. To confirm the differentiation status of DCs, we will evaluate the levels of CD11b and CD117 by flow cytometry using respective antibody from ImmunoTools. Following the loading of dendritic cells with cancer antigens via various methodologies and subsequent maturation, DCs will be administered intraperitoneally to mice. After a defined schedule of immunisations, TNBC will be induced by the administration of 4T1 cells into the abdominal mammary gland. Mice will be followed-up for cancer development. Once the mice are euthanised, the spleen and lymph nodes, as well as the tumours that may arise, will be collected from all mice. T cell populations will be characterised by flow cytometry using the antibodies anti-CD4, anti-CD8a, anti-CD25, anti-CD62L and anti-CD44 from ImmunoTools.

ImmunoTools *special* AWARD for **Ana Isabel Sebastião** includes 10 reagents

Recombinant mice cytokines: rm GM-CSF, rm Flt3L/CD13

FITC- conjugated anti-mouse CD11b, CD44, CD8a

PE - conjugated anti-mouse CD117, CD4, CD8a

APC - conjugated anti-mouse CD62L, CD25

DETAILS more AWARDS