ImmunoTools *special* Award 2022

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Establishment of rapid and high-throughput DC-T cell priming assays for validation of anti-cancer nanovaccines

Dendritic cells (DCs) are a group of antigen-presenting cells (APCs) that link innate and adaptive immune systems. DCs are specialized in processing and presenting antigen to T cells and instruct the appropriate T cells responses. In a healthy situation, DCs play a crucial role in maintaining homeostasis, by activating T cells to eliminate infected or malignant cells, or by promoting regulatory T cells to prevent chronic inflammation. In the context of cancer, they play a crucial role in eliciting anti-tumor cytotoxic $CD8^+$ T cell responses.

DCs can be broadly categorized into plasmacytoid DCs (pDCs) and conventional DCs (cDCs). While pDCs' main function is to produce type I interferon (IFN-I), cDCs are the most potent in antigen presentation and T cells activation. cDCs can be further subdivided into distinct subsets such as the DC1 and DC2, that primarily activates $CD8^+$ and $CD4^+$ T cells, respectively. Furthermore, recent single-cell technologies have allowed a deeper characterization of new DC subsets that includes DC3, with hybrid $CD14^+$ monocytes/DC2 phenotype, and $CD169^+$ Axl^+DC (See 2017, Villani 2017).

DC-based therapies have generated a significant interest for cancer immunotherapy. However, due to scarcity of DCs, initial development of DC-based therapies in cancer were prepared using enriched APCs or monocytes, which has shown improvement albeit limited clinical benefits. Recent advancement allows direct isolation of a more clinically and physiologically relevant blood-derived DCs, as well as *in situ* DC-targeting technologies. The goal of *in situ* approach is to deliver tumor antigens (ag) directly to DCs, for example using antibodies or ligands that bind to DC-specific receptors. One such receptor is CD169, in which we previously demonstrated to be an effective entry molecule to deliver antibody-based or liposome-based nanovaccines by specific targeting of $CD169^+$ Axl^+ DCs and monocytes in human (Affandi 2020, Nijen Twilhaar 2021, Affandi 2021).

However, due to low percentages of human primary DCs and ag-specific naive T cells, testing these nanovaccines often rely on using monocyte-derived DCs and TCR-transduced antigen-specific T cell clones, which does not truly reflect priming of naïve T cells by primary DCs in vivo. Recently, a simple, rapid (10d), and high-throughput assay, designed to rapidly prime naive ag-specific T cells using whole PBMCs, have been described (Bozkus 2021, Roudko 2020 Bozkus 2019). This method relies on both primary DCs and naïve T cells, thereby fully recapitulating DC priming of ag-specific T cells in vivo. Here, we aim to establish and utilize this novel method to investigate whether our nanovaccines can effectively prime tumor ag-specific T cells ex vivo.

In this proposal our objective is to establish a rapid PBMC-based immunogenicity assay to investigate nanovaccines efficacy to prime ag-specific T cells, with the following specific aims:
1. Development of T cell priming and expansion assay using bulk PBMCs.
2. Targeting and activation of DC subsets by nanovaccines, including antibody-based and liposome-based platforms
3. Immunophenotying of antigen-specific activated CD8 and CD4 T cells.

As an alternative, we will establish CD34-derived DC method based on previous work from our current collaborator Guermonprez lab (Anselmi 2020). The advantage of this approach is the generation of all human DC subsets including DC1, DC2, DC3, $CD169^{+}$ Axl^{+} DCs and pDCs. This will allow us to study our nanovaccines on DC biology in great details. Furthermore, we are in the process of collecting PBMCs from both healthy individuals and patients with melanoma and gastrointestinal malignancies. The development of both assays in our current laboratory will help us to achieve our goal to develop and to validate our ‘off-the-shelf’ vaccine platforms for cancer therapy using clinically relevant materials.