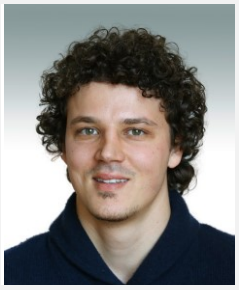
ImmunoTools *special* Award 2015



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Characterization of vaccine Dendritic cells

Dendritic cells (DCs) are key players in the initiation of adaptive immune responses. DCs recognize danger signals coming from microbes, transformed or dying cells, via a plethora of pattern recognition receptors that drive the phenotypic maturation of the DC into a cell equipped to initiate an immune response tailored to each invading threat. Matured DCs present pathogen or tumor derived antigens in a balanced context of immune stimulatory surface receptors and secreted cytokines that together instruct other immune cells and shape the resulting immune response. This unique capacity is exploited in DC-based cancer immunotherapy where autologous tumor antigen loaded DCs are used as therapeutic vaccines. Previously, we used *ex vivo* generated monocyte derived dendritic cells (moDCs) but with limited clinical success. More recently, we have started to exploit a naturally occurring DC subset directly isolated from patient blood for immunotherapy in late stage melanoma patients, the plasmacytoid DC (pDC).

First results indicate a clear clinical advantage for pDC vaccinated patients over patients receiving standard chemotherapy or moDCs. At this moment it is unclear what makes pDCs so successful as compared to moDCs. Thus the intriguing question that now arises is: What makes pDCs so successful and stand out from previously used moDCs? Based on the known ability of pDCs to affect the function and immunogenicity of other immune cells, we hypothesize that vaccine pDCs express and secrete proteins that instruct other cells to participate in the anti-tumor immune response. Our data so far demonstrates these proteins may not be the usual suspects known from moDC based research, e.g. the costimulatory molecules CD80 or CD86. Therefore we need an unbiased approach to bring forward novel candidates that determine pDC success.

To identify these, I use mass spectrometry-based proteomics to analyze the surfacome and secretome of vaccine pDCs. The expression of ligands or receptors for the proteins identified with mass spectrometry, on other immune cells, will focus

our subsequent search for cells collaborating with pDCs. To find these cells we will exploit several different *in vitro* assays to directly asses which immune cells interact with vaccine pDCs as compared to vaccine moDCs. Besides a way to test the role of proteomics derived pDC specific proteins, these in vitro assays are already a secure and stand alone source of relevant data on the cellular targets of pDCs. In combination with these findings, I want to perform *in vitro* assays to confirm these results. Furthermore the use of recombinant cytokines and chemokines of interest could help us to understand the role of pDCs upon stimulation and their communication with other immune cells.

Finally, we are in the unique position to test our key findings on lymph nodes from pDC vaccinated patients. In these lymph nodes we can trace back vaccine pDCs, verify effector cells and demonstrate the local presence of predicted molecular players. Together, proteomics data, *in vitro* assays and *in vivo* lymph nodes will provide us with the great opportunity to decipher the molecular and cellular mechanism behind the success of pDCs in cancer immunotherapy. This knowledge is of the utmost importance to control and further develop DC based cancer immunotherapy.

Furthermore, our interest is to understand the migration process of dendritic cells depending on the clinical grade stimuli we are using. These results will give us information about, where each subset is most likely to migrate and how they interact with other immune cells, e.g. T cell priming. I want to test a number of chemokines and cytokines and proof which effect this has on the dendritic cells and other immune cells.