Cancer vaccine strategies to improve immunotherapy: many roads lead to Rome

Tondini, E.

ENGLISH SUMMARY

The immune system has evolved to guard our body and to remove anything that represents a threat to our health, such as pathogens that cause infections or diseases, or tumor cells. The immune response is orchestrated by a sophisticated network of immune cells and molecules which interact with each other to initiate and execute an immune reaction.

An immune response develops over several controlled steps. Activation of the immune system requires a combination of multiple distinct signals to properly stimulate effector cells. After execution of the immune reaction, which may take from several days up to several weeks, several mechanisms are activated to dampen and inhibit the immune cells. This down-regulation is essential because the ongoing activation of the immune system may have pathological consequences if left uncontrolled.

It was recently realized that during cancer pathogenesis these regulatory mechanisms also represent escape strategies adopted by tumor cells. In fact, the immune system has the intrinsic potential to recognize and attack tumor cells, however most cancers evolve ways to suppress this.

Cancer immunotherapy is a blooming branch of medical sciences aimed at potentiating anti-tumor immunity and reverse immune suppression. It led to the development of novel treatments that have already become part of standard care for different cancers (such as immune checkpoint inhibitors) but many additional potential treatments are under investigation, such as therapeutic cancer vaccines discussed in this thesis.

Therapeutic cancer vaccination is aimed at raising an immune response against tumor-specific proteins. This response is mediated by T lymphocytes, or T cells, which can be instructed to recognize and kill tumor cells based on the cancer-specific protein antigen provided by the vaccine. This therapy holds the potential to specifically attack tumor cells leaving healthy cells unharmed. However, the employment of cancer vaccines has been so far limited by lack of therapeutic efficacy. This can be attributed to suboptimal vaccine formulations and to immune suppression at the tumor site.

In this thesis, different approaches were explored in pre-clinical setting to increase the potency of cancer vaccines by improving the way the vaccine is formulated and delivered. In **chapters 2** and **3** a novel conjugate vaccine was presented, from its design to its synthesis and evaluation in vitro and in vivo. It was shown that the covalent linkage of peptide antigen to the potent adjuvant CRX-527, a chemically defined Toll like receptor (TLR) 4 ligand, enhanced vaccine potency compared to the classical administration of antigen mixed with this ad-

juvant. This translated into improved T cell-mediated protection in mice induced by the vaccine against an aggressive melanoma tumor .

The same concept was applied to a peptide vaccine adjuvanted by the TLR7 ligand hydroxyadenine. This adjuvant is less potent than CRX-527 but can improve the anti-tumor functions of the T cell response induced by the vaccine. An antigenic peptide was coupled to the adjuvant and a second ligand to explore the effect of dual conjugation in two different studies. In **chapter 4,** dual conjugation of hydroxyadenine and the ligand mannose 6-phosphate to antigenic peptide was explored. Intracellular targeting to the M6P receptor eventually resulted in lower activation of T cells. This demonstrated how vaccine formulation may impact vaccine efficacy by modifying intracellular routing. In **chapter 5,** it was explored the design of a peptide vaccine conjugated to two distinct adjuvants: hydroxyadenine and Pam3CysSK4, the latter being currently evaluated in the clinic for the treatment of HPV16-related malignancies. This chapter reports the validation of this newly designed and potent dual conjugated vaccine and opens the way for further pre-clinical evaluation for its anti-tumor efficacy.

Encapsulation of the vaccine in nano-sized particles is another strategy currently explored for improving vaccine potency. In **chapter 6**, dextran nanogels were evaluated as carriers of peptide vaccines. Peptides were covalently linked to these nanoparticles to protect the antigen after injection in vivo from dispersal and degradation before reaching immune cells. This resulted in the induction of higher numbers of specific T cell responses in vaccinated mice, that displayed a higher poly-functional profile, supporting the idea that nanogels represent a suitable carrier to potentiate the delivery of peptide-based vaccines.

The rapid development of sequencing and high throughput technologies of the last decades has allowed the identification of potential tumor antigens at high resolution of cancer cells collected from a single patient. Personalized cancer vaccines are the new frontier of cancer vaccine research, and this concept was explored in mice. In **chapter 7**, a new design for a DNA cancer vaccine was presented. DNA vaccines represent a flexible, cost effective and easily manufactured platform suitable to meet the demands of personalized cancer vaccines. Moreover, this chapter shows how separate immunotherapies such as cancer vaccines