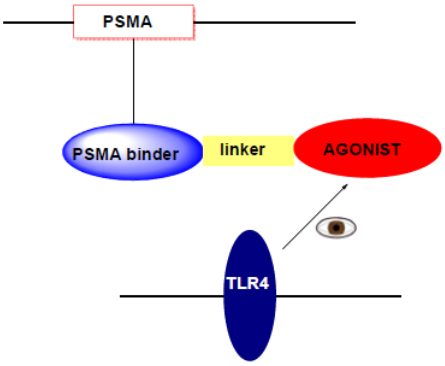
1. Publishable summary

Summary description of the project objectives

The immune system is a pivotal element in the defense of the organism against microbial infections as well as in the control and surveillance of malignant neoplasms. Immune cells scan tissues with the objective of removing newly malignant cells before they turn into fully formed tumors. Innate immune cells recognize the intruding pathogen and trigger appropriate immune response with the help of Toll-Like receptors. They are expressed in sentinel cells such as macrophages and therefore they are responsible for the *macrophage* activation and control of parasitic infections. Among all the Toll family receptors, TLR4 recognizes a wide array of ligands, including lipopolysaccharide (LPS) a cell wall component of Gram-negative bacteria, responsible for macrophage activation and signal transduction.

In recent years there has been evidences indicating that mutations in TLR4 were associated with risk of prostate cancer. Prostate cancer represents one of the most common cancers diagnosed in males in the Western countries. Standard pharmacological therapy, consisting of ablation of androgens, is initially efficient, but most treated patients progressively develop the disease again and eventually die of cancer. Consequently, many efforts are being made to identify novel targets and agents useful for the treatment of this disease. A notable discovery in recent years has been the identification of an over-expressed protein in the surface of the prostate cancer cells, namely, prostate specific membrane antigen (PSMA).

The main objective of the project TLRPROSPATE is to chemically program the TLR4 receptor so that it signals when it detects a prostate antigen. Thus, designing a bifunctional linker carrying a small molecule PSMA binder on one side and a weak agonist on the other side would allow the macrophages to sense it thinking to be a bacterium and kill the dispensable tissue.



Description of the work performed since the beginning of the project

During the first year of TLRPROSTATE, attempts to synthesize the target molecule were carried out. However, due to the high complexity of it, different strategies in order to perform the synthesis had to be explored. Synthesis of PSMA inhibitor were carried out using the urea based approach, cwhile the agonist derived from monophosphoryl lipid A was synthesized using the proposed approach. The dipeptide PSMA approach was not

successful due to the instability of the diazo group, which was light sensitive and not stable. In fact, it decomposed after few minutes of being synthesized.
Once the molecule was obtained, during the second year stability studies were performed using human serum followed by binding studies to prostate cancer cells. Also, binding studies have been carried out in order to show the efficacy of the compound. Cell proliferation assays were performed to check the suitability in its inhibition of cancer cells. Finally, IC50 values were measured using the Naaladase Assay.

Description of the main results achieved so far

The synthesis of the main molecule described in TLRPROSTATE has been achieved in a rather reliable manner since we are able to obtain 1 g of it. Stability of the product has been tested in human serum at 3 micromolar in concentration, showing its degradation after 18 hrs, which points out its stability in serum. Moreover, the expression and purification of the PSMA protein allowed us to study by NMR its interaction with the protein showing its efficacy.

Expected final results and their potential and use

Prostate cancer is the second most common cancer worldwide for males, and the fifth most common cancer overall. Within the 27 countries of the European Union, prostate cancer has emerged as the most frequent cancer amongst men, with increasing rapidly over the past two decades. With the results obtained so far, we are extremely confident that the development of synthetic vaccines can help scientists and researchers to prevent the reoccurrence of potentially fatal diseases. Using the body's own immune defenses, this new treatment option could save millions of lives and reduce the cost of treatment.
In this regard, this project may have a profound impact on the future prostate cancer research. It will significantly contribute to the effort of finding a cure for prostate cancer as well as understanding the mechanisms of action.