# Chemical programming of Toll like Receptor 4: Design, synthesis and biological studies of prostate-cancer vaccines

## Rapports

Informations projet

**TLRPROSTATE**

N° de convention de subvention: 623155

Projet clôturé

**Date de début**
5 Janvier 2015

**Date de fin**
4 Janvier 2018

**Financé au titre de**
Specific programme "People" implementing the Seventh Framework Programme of the European Community for research, technological development and demonstration activities (2007 to 2013)

**Coût total**
€ 341 387,10

**Contribution de l'UE**
€ 341 387,10

**Coordonné par**
AGENCIA ESTATAL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Spain

## Ce projet apparaît dans...


# Final Report Summary - TLRPROSTATE (Chemical programming of Toll like Receptor 4: Design, synthesis and biological studies of prostate-cancer vaccines)

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.
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, while 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.

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.

1. Synthesis of the dipeptide inhibitor as well as the urea based inhibitor was made. While the dipeptide inhibitor was synthesized according to what was previously reported, in the case of urea based inhibitors slight modifications were made in order to assure its efficacy when making in vitro assays. To it, it was added a maleimide and aromatic moieties that will allow the PSMA to bind and remain fixed on the surface of the prostate cancer cell.

2. On the other hand, the synthesis of the agonist was carried out as it was planned.
3. Final purification of the molecule using reverse phase techniques was performed in order to achieve a reliable purity of the compound.
4. Stability assays of the final molecule were carried out in human serum at a concentration of 3 micromolar. A portion of it was degraded after 18 hrs, which clearly shows enough stability of the molecule.
5. Binding specificity studies were performed using both positive and negative PSMA cells. Following the procedure described in Annex I we got a preliminary binding constant of 8 nM, which considerably enhances the previous results reported in literature.
6. We carried out a Naaldase Assay in order to get IC50 values for the compound. However, no significant results have been observed using this technique.
7. PSMA protein was expressed and purified following standard protocols, and the binding of the protein with the molecule was checked by NMR techniques, showing its efficacy.
8. Cell proliferation assay was carried out. This showed an important inhibition of the PSMA positive cells, which constitutes a promising result of the utility of the product.
9. Attempts to develop animal models are currently under way.

A summary of the progress of the researcher training activities/transfer of knowledge
At TSRI under the supervision of Prof. Kim D. Janda, Dr. Miranda has complemented his expertise in basic design of vaccines, being trained in the use of new techniques such as:
- Surface Plasmon Resonance and Isothermal titration calorimetry which are widely used in the measurement of binding constants.
- Field effect transistor which constitute a new technique and a new approach on the measurement of binding constants.
- Protein NMR, which is an important tool to study disposition of disorder proteins. This was able thank to a collaboration with the group of the Nobel’s Prize laureate Kurt Wuthrich. This technique was used to detect the binding affinity of the PSMA antigen.

- Circular dichroism, to analyze the structure of the PSMA protein expressed.
- Reverse phase HPLC techniques, which are highly important in order to get pure products able to go to in vivo studies.
- Cell culture techniques, which has been highly useful in culturing the corresponding cell lines required.
- Cell binding assays which in which is required to work with radioactive compounds.
- Enzyme-Linked ImmunoSorbent Assay (ELISA), to detect the presence of antigens in blood. This technique will be highly useful in future experiments for studying the in vivo efficacy of TLRPROSTATE.
Highlight clearly significant results
A new synthetic approach has been developed for the synthesis of the final molecule of TLRPROSTATE. With this new synthetic route, we can skip up to 4 steps comparing with other different synthesis published in literature. Moreover, an important difference from the reported procedures in literature is the possibility of scaling up the amount of material needed. Thus, we have been able to synthesize up to 1 gr of target molecule, which constitutes an important outbreak in order to proceed and make in vivo experiments. In vitro stability experiments have shown that product was degraded after 18 hrs incubation at 37 C in human serum. In accordance with this result, the final compound was regarded as being stable. Also, the in vitro efficacy of the product was studied by Naaladase Assays and NMR techniques. Binding to PSMA was detected and measured, obtaining IC50 values.

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.

**Dernière mise à jour:** 26 Juillet 2018

**Permalink:** https://cordis.europa.eu/project/id/623155/reporting/fr