Principal Investigator: Francisco Corzana

Grant Title: Carbon Dots-MUC1 conjugates: synthesis and applications of a novel class of fluorescent self-adjuvating cancer vaccines.

Abstract.

Prof. Francisco Corzana

Therapeutic vaccines are attracting considerable attention as potential weapons to fight cancer through the immune system. Mucin 1 (MUC1) is a glycoprotein found on the surface of epithelial cells. While in healthy cells the peptide backbone is decorated with complex carbohydrates, in cancer cells this molecule is overexpressed and presents truncated and simple carbohydrates such as the Tn antigen (α-*O*-GalNAc-Ser/Thr) that is exposed and can be recognized by the immune system. However, MUC1-based vaccines have low *in vivo* stability and are poorly immunogenic. To enhance the immune response, we prepared a set of chemically modified MUC1 derivatives bearing unnatural amino acids to enhance the antigen stability and trigger a stronger immune response. Moreover, the multivalent presentation of antigens on the surface of protein carriers or nanoparticles can improve the MUC1 immunogenicity. In this project, we explore the use of carbon dots (CDs) nanoparticles as novel set of carriers with inherent immunostimulant properties to enhance the immune response. CD-MUC1 conjugation is expected to improve antigen stability and cellular internalization properties in antigen-presenting cells. Additionally, the implementation of CDs in combination with chemically modified MUC1 variants is expected provide a novel class of nanomaterials with high immunogenicity and high *in vivo* stability for cancer vaccines.

Objectives.

The overall aim of this project is to synthesize, characterize and evaluate a novel class of self-adjuvating cancer vaccines based on unnatural MUC1 derivatives supported on CDs.

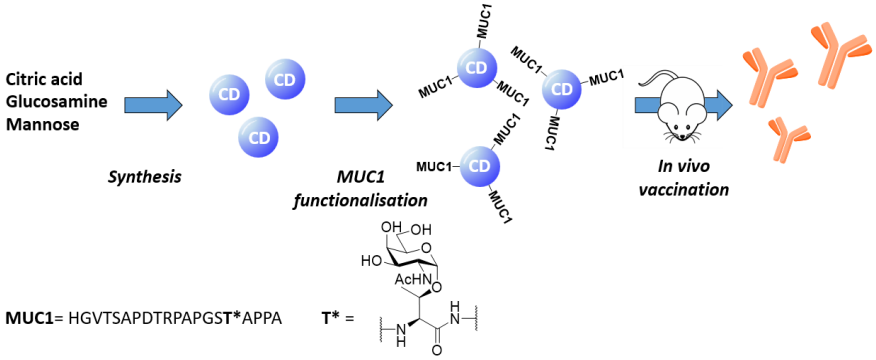
The specific objectives of this project include: i) synthesis of unnatural MUC1 derivatives; ii) synthesis of CDs; iii) chemical conjugation and characterization of CD-MUC1 conjugates; iv) *in vivo* evaluation of the immune response and tumor growth suppression studies.

Methods used.

CDs were prepared from citric acid, glucosamine, and mannose precursors following reported procedures.[1] The chemically modified MUC1 variants were prepared *via* standard organic synthesis and assembled in the peptide structure through solid phase peptide synthesis.[2] The biological evaluation of CD-MUC1 conjugates was performed through cellular internalization assays, SPR studies with anti-MUC1 antibodies, and *in vivo* vaccination in mice models.

Results.

The CDs and modified MUC1 variants were successfully synthesized and coupled together to form the corresponding CD-MUC1 conjugates and the MUC1 loading was measured *via* NMR analysis showing an average MUC1 load of ~30% w/w. Initial studies *in vivo* demonstrated that the CD-MUC1 conjugates are non-toxic. Preliminary *in vivo* assays demonstrated that the CD possess good immunostimulatory properties and were able to elicit an immune response toward the MUC1 antigen.



Synthesis and evaluation of CD-MUC1 conjugates

[1] a) S.A. Hill, *et al., Nanoscale*, **2016**, *8*, 18630-18634. b) M. Ghirardello, *et al., Nanoscale Adv.,* **2022**, *4*, 1770-1778. c) Q. Zhou, *et al., ACS Nano*, **2021**, *15*, 2929-2932.

[2] V.J. Somovilla, *et al., J. Am. Chem. Soc.,* **2017**, *139*, 18255-18261.