UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.19

Proponent: Prof. Francesco Peri

Project Title: Overcoming Challenges in Drug Delivery: Design and Biological Evaluation of Liposomal Formulations of bioactive molecules for Cancer Immunotherapy and Fibrosis Treatment.

Scientific background and 'open issues'

Toll-like receptor 4 (TLR4) is a key pattern recognition receptor in the innate immune system, responsible for initiating signaling cascades that lead to the activation of immune responses and the production of pro-inflammatory cytokines and chemokines.

In last 15 years our research group developed new molecules able to modulate the activity of TLR4 by stimulating (agonists) or inhibiting (antagonists) it.

We have developed several generations of **TLR4 agonists** and we are collaborating with the big pharma Croda to bring to clinic and to market some of these agonists as vaccine adjuvants. On the other hand, TLR4 agonists have been shown to induce the M2 to M1 macrophage switch, which is crucial for enhancing antitumor immune responses. The M2 to M1 switch can lead to increased immune cell infiltration, enhanced antigen presentation, and improved cancer cell elimination. However, the optimal formulation and delivery strategies for TLR4 agonists in cancer immunotherapy remain to be elucidated.

TLR4 antagonists can inhibit the activation of TLR4 signaling pathways, thereby mitigating inflammation and tissue damage. Fibrosis is a pathological condition characterized by excessive deposition of extracellular matrix (ECM) components, leading to tissue scarring and organ dysfunction. TLR4 signaling has been implicated in the pathogenesis of fibrosis, as it contributes to the activation of fibroblasts and the production of pro-fibrotic cytokines. Thus, TLR4 antagonists have emerged as potential therapeutic agents for managing fibrosis in various organs, including the liver, lung, and kidney. However, further research is needed to optimize the formulation and delivery of TLR4 antagonists for fibrosis management.

Despite the promising preliminary results, there are still gaps in our understanding of the optimal formulation and delivery strategies for TLR4 modulators. This project aims to address these gaps by **developing and evaluating liposomal formulations of TLR4 modulators for cancer immunotherapy and fibrosis management**, respectively. The proposed research will contribute to the advancement of TLR4 modulator-based therapies and provide valuable insights into the design of effective formulation and delivery strategies for these modulators.

Objectives

The project aims to advance our understanding of the optimal formulation and delivery strategies for TLR4 modulators, ultimately enhancing their therapeutic potential in cancer immunotherapy and fibrosis management. Objectives:

1) To develop and characterize TLR4 modulator liposomal formulations
2) To investigate the potential of **TLR4 agonist formulations** in inducing M2 to M1 macrophage switch and stimulating immune responses against cancer cells.
3) To evaluate the therapeutic efficacy of **TLR4 antagonist formulations** in mitigating fibrosis-induced tissue damage and inflammation.

By accomplishing these objectives, we will provide valuable insights into the design of effective TLR4 modulator-based therapies related to inflammation and anti-inflammation.

Methodologies

This project will employ a combination of techniques to achieve the proposed objectives. The methodologies include:

1) **Synthesis and characterization of TLR4 modulator liposomal formulations:** We will synthesize and characterize various TLR4 modulator liposomal formulations using thin-film hydration, extrusion, and other established methods. The formulations will be characterized for their physicochemical properties, such as particle size, polydispersity index, zeta potential, and encapsulation efficiency.
2) **In vitro evaluation of TLR4 modulator formulations:** We will assess the immunomodulatory effects of the TLR4 modulator formulations using in vitro assays. For TLR4 agonist formulations, we will evaluate their ability to induce the M2 to M1 macrophage switch and stimulate immune responses against cancer cells using flow cytometry, enzyme-linked immunosorbent assay (ELISA), and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to analyze cytokine and surface marker expression. For TLR4 antagonist formulations, we will investigate their ability to inhibit fibroblast activation and pro-fibrotic cytokine production using similar techniques.
3) **In vivo evaluation of TLR4 modulator formulations:** We will evaluate the therapeutic efficacy of the TLR4 modulator formulations in relevant animal models. For TLR4 agonist formulations, we will use tumor-bearing animal models to assess their ability to induce anti-tumor immune responses and inhibit tumor growth. For TLR4 antagonist formulations, we will use fibrosis-induced animal models to evaluate their impact on fibrotic processes and tissue damage. We will evaluate the underlying molecular mechanisms by analyzing the gene expression profiles using western blotting, and qRT-PCR.

The general project design will involve an iterative process of formulation development, characterization, and evaluation, with continuous refinement based on the results obtained. This approach will enable us to identify the most promising TLR4 modulator formulations and provide valuable insights into their therapeutic potential and mechanisms of action.

Collaboration / Co-tutoring opportunities

The project will benefit from intradepartmental collaborations with experts in immunology, cancer biology, and fibrosis research.

A first doctorate position is available to develop innovative formulations for antitumor immunotherapy in collaboration with Dr. Giovanna D'Amico (San Gerardo Hospital, Monza). A second doctorate position is available to develop antagonist formulations targeting fibrosis in collaboration with Dr. Sara Lovisa (Humanitas University, Milano).
Important: both PhD students will be followed by post-docs with expertise in chemistry (Dr Alessio Romerio, Assegno di Ricerca), formulation and nanotechnology (Dr Monsoor Shaik, Assegno di Ricerca), biology and immunology (Dr Alice Italia, PhD student.

Project's Sustainability & Mobility

Coherence
The proposed project aligns with the lab's expertise in TLR4 modulator research and liposomal formulation development. The group of F. Peri is developing innovative TLR4 agonists and antagonists that have been validated at preclinical stage (in vitro and in vivo activity). F. Peri published more than 70 papers on the discovery and characterization of new TLR4 targeting molecules, he is an internationally recognized expert in Medicinal Chemistry and TLR4 modulation by chemical means.

5 recent publications authored by the Proponent's research group:
1) Overcoming Challenges in Chemical Glycosylation to Achieve Innovative Vaccine Adjuvants Possessing Enhanced TLR4 Activity A Romerio, AR Franco, M Shadrick, MM Shaik, V Artusa, A Italia, F Lami, **2023**, ACS omega 8 (39), 36412-36417
2) New glucosamine-based TLR4 agonists: design, synthesis, mechanism of action, and in vivo activity as vaccine adjuvants A Romerio, N Gotri, AR Franco, V Artusa, MM Shaik, ST Pasco, U Atxabal, **2023**, Journal of Medicinal Chemistry 66 (4), 3010-3029
3) Synthetic glycolipids as molecular vaccine adjuvants: mechanism of action in human cells and in vivo activity FA Facchini, A Minotti, A Luraghi, A Romerio, N Gotri, A Matamoros-Recio, **2021**, Journal of Medicinal Chemistry 64 (16), 12261-12272
4) Effect of chemical modulation of toll-like receptor 4 in an animal model of ulcerative colitis
FA Facchini, D Di Fusco, S Barresi, A Luraghi, A Minotti, F Granucci, European Journal of Clinical Pharmacology 76, 409-418
5) Increasing the chemical variety of small-molecule-based TLR4 modulators: An overview
A Romerio, F Peri **2020**, Frontiers in immunology 11, 538206.

Mobility
Putative foreign institutions for student's mobility:
1) CIC bioGUNE, Spain. Dr Juan Anguita: in vitro and in vivo tests; Prof. Jesus Jimenez-Barbero: physical chemical methods for characterizing nanostructures
2) Anglia Ruskin University, Cambridge, UK. Dr Grisha Pirianov: in vitro and in vivo tests
3) S. Vogel (University of Maryland, USA): in vitro and in vivo tests on TLR4 antagonists