

# Title: Development of cancer vaccines against plasticity antigens to overcome therapeutic resistance in lung cancer

PI: Arianna Palladini

Location: Department of Molecular Medicine, Cancer Vaccines and Immunotherapy Laboratory (CVIL)

Research theme / topic: Cancer vaccines in Lung Cancer

Lung cancer is the leading cause of cancer death worldwide and non-small cell lung cancer (NSCLC) accounts for approximately 80% of cases. About 70% of patients with NSCLC have advanced disease at diagnosis. Despite the progresses in the treatment of NSCLC, the prognosis remains poor, with an estimated five-year overall survival (OS) of 16%. The introduction of targeted therapies and immunotherapy in clinical practice has redefined treatment options for this disease. Nevertheless, the onset of drug resistance is still the major obstacle to treatment success.

Immunotherapy aims to mobilize the immune system to destroy tumor cells. Immune checkpoint inhibitors (ICIs) have been developed with the intent to act on those pathways of "self-tolerance" (e.g., PD-1/PD-L1) aimed at physiologically controlling auto-reactivity, used by tumors to escape recognition by the immune system.

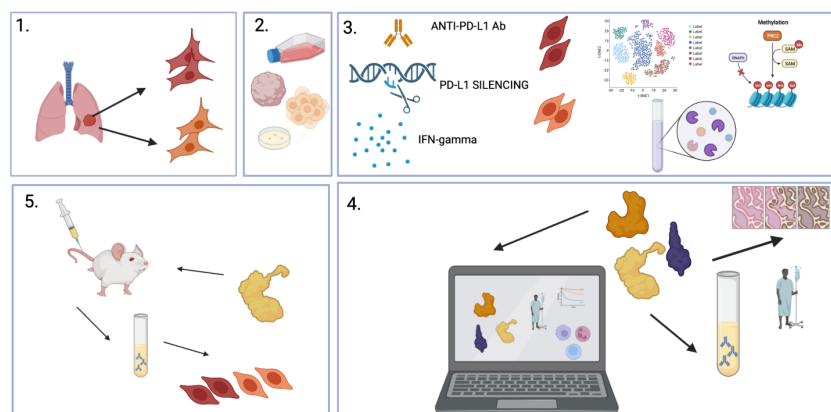
Many patients still do not experience clinical benefit upon these therapies (hyperprogressors and fast progressors). On the other hand, an exciting advantage of novel immunotherapeutic strategies is the durability of clinical benefit, with some patients achieving disease control for many years even after the discontinuation of treatment. No validated clinical or molecular predictors of fast progression and hyperprogression have been identified because of the heterogeneity and retrospective nature of the studies, although some involved factors have been suggested (Angelicola et al., 2021).

Cancer vaccines represent a very promising strategy to both prevent tumor onset in high-risk patients and to provide a therapeutic alternative to chemotherapy and targeted therapy for patients experiencing relapse or with no other treatment options. Nowadays, about twenty cancer vaccine antigens are under evaluation in clinical trials (Palladini et al., 2018; De Giglio et al., 2021). The combination of cancer vaccines with ICIs could result in a boost of immune stimulation, after the tumor-induced prime stimulation, together with an awakening of T lymphocytes (Liao and Zhang, 2021).

PD-L1 is involved in stem cell differentiation and cancer cell plasticity (Kuo et al., 2021; Takahashi et al., 2019; Xiang et al., 2022). Based on this evidence, the first goal of this proposal is the evaluation of how ICI treatment affects NSCLC plasticity, by influencing PD-L1 signalling pathway. The second aim of this proposal is the identification and validation of molecules involved in ICI-induced plasticity as candidate antigens for cancer vaccine development.

## Experimental plan

1. Characterization of normal lung epithelial cells and a panel of human NSCLC cell cultures
2. Establishment of 2D-growth and 3D-growth (spheres, soft agar colonies and organoids) cultures
3. Molecular characterization of the cellular adaptation induced by ICI treatment/PD-L1 modulation and IFN gamma
4. Membrane antigens modulated by PD-L1 will be validated as candidates for cancer vaccines through *in silico* vaccination experiments. The expression level of the identified candidate antigens will be tested on tumor biopsies at the baseline and, if available, at the progression. Sera of patients long-responders to immunotherapy will be analysed to evaluate the presence of autoantibodies reactive to previously identified membrane antigens.
5. The immunogenicity of the two best-fit candidate antigens will be evaluated *in vivo*. Mice sera will be collected and sera activity will be tested on *in vitro* models.



**Techniques.** Cell cultures: achievement of primary cultures from tumor biopsies, 2D and 3D growth tests, transfection, silencing, cloning. Molecular analysis: confocal microscopy, immunofluorescence, flow cytometry, sorting, Real-Time PCR, Western Blot, ELISA test, ELISPOT. In silico analysis will be performed in collaboration with Prof. Francesco Pappalardo (Combine group, University of Catania). Dr. Francesco Agustoni (University of Pavia and IRCCS San Matteo Hospital) will provide clinical data, tumor biopsies and sera.

Palladini et al. Oncoimmunology 2018; Angelicola et al., Cancers 2021; Ruzzi et al. TCR 2022