REPORT
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MODULATION OF GLYCEMIA AS A STRATEGY TO IMPROVE THE BENEFIT OF CHEMORADIOTHERAPY AND IMMUNOTHERAPY IN NON-SMALL-CELL LUNG CANCER

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Non-small cell lung cancer (NSCLC) is related to various metabolic alterations. On the one hand, the metabolism of lung cancer cells is different from the metabolism of non-transformed lung cells. On the other hand, the patient's blood glucose and diabetes have an impact on the incidence of lung cancer and also on the clinical outcome of chemo-radiotherapy treatment.

In this project financed by La Fundació La Marató de TV3 we propose the following hypothesis:

1) Patient metabolism and serum metabolite levels are related not only to the incidence and development of tumours (as described in the case of diabetes) but also to responses to chemo-radio-immunotherapy. Therefore, the analysis of certain metabolites could be useful to stratify the prognosis of patients, and by focusing on systemic metabolism, it would also improve the clinical outcome.

2) Conversely, we also propose, based on extensive evidence, that the tumour itself secretes metabolic hormones and cytokines that alter host metabolism and that may promote unfavourable metabolic and nutritional phenotypes. In these cases, the molecules released by the tumour can become possible therapeutic targets.

Therefore, group 1 of the coordinated project had the objective of following a group of lung cancer patients from the moment of diagnosis until after treatment, and for years afterwards, to be able to know a posteriori:

1) whether there were metabolites or cytokines in the patients' blood that could be used to predict whether the patient could obtain a benefit from chemo/radiotherapy and subsequent immunotherapy.

2) if changes in weight, glucose and other metabolic parameters occurred could indicate that the tumour was affecting the patient's metabolism and that this could affect the outcome of the therapy. Furthermore, these changes could be amenable to clinical intervention.

The other group (group 2) would find out whether high glucose levels like those seen in diabetes would cause, in animals, a decreased response to chemotherapy.

2. Results

Group 1

After the difficulties experienced during the first years of the project (due to the COVID stoppage in pulmonology and thoracic oncology), we were able to begin the prospective collection of blood samples, as well as tumour biopsies. At the end of the project, 46 patients have been recruited, of which we have follow-up blood samples(pre- and post-chemo/radio) from 24 and tumour biopsies from 6. In these sequential samples we have carried out an exploratory study of cytokine levels using *O-Link* technology, the results of which we are analysing and hope to have ready in the coming months. We have also analysed GDF15, a cytokine previously associated with cachexia which is elevated in samples from animals with tumours.

It is important to note that 20% of the patients had a weight loss of 5-10% and 35% of more than 10% on the first visit compared to their usual weight. We have found a statistically significant correlation between GDF15 levels in the blood and the percentage of baseline weight loss (i.e., calculated using the patient's usual weight and the weight recorded at the first visit, prior to treatment with chemoradiotherapy) in the 15 patients in whom measurements of this cytokine are available. This association is maintained both in the analysis by weight loss groups and when the continuous variables are correlated.

On the other hand, we have been able to analyse different serum parameters associated with glycaemia in order to correlate them with the response to chemo-radio treatment. In addition, the changes in the levels of these markers between the first (i.e., before treatment) and the second follow-up visit (i.e., after treatment) have also been evaluated. As representative examples, a slight increase in insulin and C-peptide is observed in non-diabetic patients with treatment and higher levels of insulin and glycosylated haemoglobin (HbA1c) could be associated with a worse response. It must be considered that many of the patients with a partial response, which are the majority, will relapse in a few months and this will surely allow us to better evaluate the impact of metabolic parameters on the response to treatment. We will also have pending the association of immunotherapy with these markers and parameters (probably in 10-12 months).

It has been discovered that animals (mice) with lung cancer have difficulties maintaining an optimal metabolism: they lose weight and their blood glucose increases. This is also associated with high levels of various cytokines detected in blood (serums). In these studies we have measured serum levels of insulin and C-peptide and see how these seem to increase, especially in females in the presence of tumours. Finally, we decided to add another cohort of patients, that of the ATEZO-BRAIN clinical trial that included 40 patients with advanced lung adenocarcinoma with brain metastases treated with 1st-line chemo-immunotherapy. In this cohort we have also carried out the study using *O-Link* technology (results pending analysis), and also the analysis of the metabolite profile using mass spectrophotometry (performed by our collaborators in Haifa). The clustering analysis, using the most variable metabolites between samples, allows patients to be classified into three groups according to their metabolic profile. With this analysis, no clear associations have been found between prognosis and metabolic profiles; however, we have yet to extend the analysis to other metabolites.

Group 2

Due to the long latency of the initially proposed experimental procedure, and the heterogeneity of tumour development in the study population, which complicated the comparison of the different planned treatments, a contingency procedure was initiated based on implants of isogenic tumour lines. For this purpose, the first tumours (obtained from pure C57Bl6 background animals in July 2023) were used for the development of tumour lines. As they are derived from a C57Bl6 strain, they could be implanted in the lungs of recipient mice of the same genetic background. Prior to their in vivo use, the tumour lines obtained were genetically modified to incorporate the luciferase gene and thus allow longitudinal monitoring by image analysis (IVIS). Likewise, the experimental validation and optimization of the hyperglycaemia induction protocols in mice was carried out. Despite being protocols established in the literature, they are experiments subject to high variability that must be appropriately optimized in each animal facility.

Once the different experimental parameters were optimized, a first pilot experiment was carried out. Implantation of the tumour line was performed by injection into the tail vein. This is an established procedure that, due to blood circulation, results in colonization of the lung parenchyma. Briefly, increased tumour growth was observed in

animals treated to develop hyperglycaemia. This result would be in accordance with the worse prognosis observed in lung adenocarcinoma patients with concurrent diabetes that gave rise to the design of this subproject.

Unfortunately, tumour progression was developed with an aggressiveness that prevented the implementation of chemotherapy treatments with a sufficient duration to statistically significantly evaluate therapeutic efficacy, making it necessary to sacrifice the animals prematurely taking into account the established animal welfare criteria. This experiment was carried out with three lung adenocarcinoma lines generated independently, obtaining similar results. The alternative of injecting a cell quantity of less than 10^5 cells per animal to increase the survival of the experimental groups resulted in excessive heterogeneity incompatible with subsequent treatments.
In view of these results, we decided to implement a new orthotopic implant protocol through direct injection into the lung parenchyma. It was necessary to make various modifications (number of cells, injection angle and complementation with extracellular matrix) in relation to similar protocols previously published (PMID: 22833819). Finally, we were able to obtain individual lung lesions with a minimum evolution period of two months until reaching the humane end point. The greater extension of the experimental period is essential to perform a minimum of 4 and a maximum of 7 cycles of chemotherapy as currently performed in clinical practice. Therefore, this new experimental model is, on the one hand, compatible with the chemotherapy treatment protocols initially planned, as well as with the restoration of normal glycemia through pharmacological treatment. These experiments are currently underway and will most likely last until the end of 2024. We hope by then to have completed the objectives initially committed to in the initial proposal.

3. Relevance with possible future implications

This project has allowed us to generate synergies with the Functional Nutrition Unit and strengthen the relationship between the Thoracic Oncology clinical team, led by Dr Nadal, and the Translational-Basic team, led by Dr Muñoz-Pinedo. Furthermore, the results generated in this project allow us to lay the foundations for future projects and participate in future competitive calls to expand the cohorts of patients with thoracic

tumours that will be characterized from the metabolic, inflammatory and body composition point of view.

We have observed that patients with higher insulin levels, whether diabetic or not, have a more unfavourable clinical behaviour. On the other hand, animals with lung cancer develop hyperglycaemia, hyperinsulinaemia, and insulin resistance. Follow-up of the clinical cohort suggests that this phenomenon could also occur in patients, but follow-up is still limited due to the delay in activation of the prospective cohort due to the COVID19 epidemic. Virtually all the patients we proposed in the proposal have been recruited, but some patients are still in the early stages of treatment and do not have a minimum follow-up to be able to perform an analysis of progression-free survival or overall survival. When we have greater follow-up, progression events can be correlated with different metabolic, inflammatory or body composition markers to determine which factors are associated with earlier tumour progression.

We anticipate that some metabolic alterations that we have detected in this project, such as hyperglycaemia or hyperinsulinaemia, can be addressed in future studies with a more interventional design where antidiabetic therapy can be intensified, or even administering metformin to patients with dysglycaemia, prediabetes and/or or hyperinsulinism without a history of diabetes mellitus or increase physical activity in patients at risk of sarcopenia and/or cachexia, or already in clinical stages of these two diseases. This possibility of performing relatively simple and inexpensive interventions could contribute to better health outcomes and improve the quality of life of patients with resectable stage III lung cancer who receive treatment with concurrent chemoradiotherapy with curative intent and consolidation with durvalumab if tumours have positive PD-L1 expression. Despite the advances achieved in recent decades, only a third of patients will be alive and free of tumour progression 5 years after treatment. Multiple therapeutic strategies are being investigated (intensification or earlier initiation of immunotherapy, new combinations, etc.) which at the moment have not shown a clear increase compared to standard treatment. New approaches that are feasible, cost-effective and that can have a positive impact on health outcomes and that are independent of the pharmaceutical industry are therefore very necessary.

4. Scientific bibliography generated

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