TÍTULO:

G (glucacon)_**L** (like) peptide-1receptor **A**gonist in **TrI**ple **N**egative Breast Cancer: **G_LATiN Study**

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**Translational Research approaches will be carried out at the MD Anderson Foundation in Madrid, in close collaboration with the IIBM (CSIC & UAM).*

Duración aproximada: 2 años

Introduction and rational

Breast Cancer (BC) is the most common malignancy among women and the second most prevalent cancer worldwide. In 2022, an estimated 2.3 million women were diagnosed with BC, resulting in approximately 670,000 deaths globally [1]. Triple-negative breast cancer (TNBC) represents approximately 10-15% of all BC cases and continues to be the BC tumour subtype with the worst prognosis. Incorporating immune checkpoint inhibitors (ICIs) into neoadjuvant protocols for TNBC has markedly improved treatment outcomes. Administering ICIs alongside standard neoadjuvant chemotherapy increases rates of pathological complete response (pCR) and improves event-free survival and overall survival [2].

However, **obesity** is even more prevalent worldwide than BC. According to un updated report by the World Health Organization (WHO) approximately 1 in 8 individuals worldwide live with obesity [3]. In fact, in 2022, 2.5 billion adults aged 18 years and older were overweight, including over 890 million adults who were obese. Obesity is a chronic, multifactorial disease characterized by excessive fat accumulation that poses significant health risks. It is assessed using body mass index (BMI), which is calculated by dividing weight in kilograms divided by height in meters square. Thus, a BMI ≥ 30 kg/m^2 indicates obesity, while a BMI between 25 and 29.9 kg/m^2 is considered overweight. Moreover, obesity induces a chronic low-grade inflammatory state associated with multiple metabolic and immune disorders. This condition is sustained at several levels within the host, including intracellular metabolic imbalance, which is regulated by various factors such as cytokines, activation of specific signaling pathways, and disruption of the balance between cell proliferation and death. In particular, pyroptosis, a form of proinflammatory programmed cell death, plays a central role in the activation and regulation of the inflammasome pathway. **Pyroptosis**, driven by the activation of Gasdermin (GSDM) proteins, promotes cytokine secretion and may represent a key mechanism underlying the metabolic dysregulation observed in obesity [4]. Although obesity and its associated complex molecular alterations have been linked to cancer risk in several tumor subtypes [5], the relationship between breast cancer (BC) and obesity remains controversial and not fully understood. Nevertheless, given the high prevalence of both BC and obesity, their coexistence is unsurprisingly common.

Indeed, the negative impact of obesity on BC outcomes has been well-documented. Thus, a 2014 meta-analysis of 82 studies evaluating the relationship between body weight and early breast cancer outcomes demonstrated a 35% increase in BC-related mortality and a 41% increase in overall mortality in women with obesity at the time of BC diagnosis compared to normal-weight BC women [6]. Additionally, overweight or obesity in breast cancer patients are less likely to

achieve a pCR after neoadjuvant chemotherapy than their normal-weight counterparts (OR = 0.80, 95% CI 0.68–0.93, P = 0.0053) [7]. Although the molecular mechanisms are not fully understood, emerging evidence suggests that increased BMI is associated with worse BC outcomes: high estradiol levels can stimulate the proliferation and survival of estrogen receptor-positive BC cells [6]. Moreover, insulin resistance and the resulting hyperinsulinemia, along with elevated insulin-like growth factors (IGFs), stimulate mitogenic signaling (PI3K/Akt/mTOR and MAPK), which promotes tumor growth and survival. Imbalances in adipokines may result in high leptin levels and low adiponectin levels which activate different signaling pathways (JAK/STAT, MAPK, and PI3K/Akt) involved in the maintenance of cancer cell proliferation, angiogenesis, and metastasis as we as low-grade chronic inflammation.

Considering all the above, evaluating whether weight loss is associated with a better outcome in early-stage BC patients is necessary. er. Data from a large prospective study of postmenopausal women in the Women's Health Initiative (WHI) Observational Study (OS) showed that intentional weight loss was linked to a lower overall risk of obesity-related cancers [7]. However, data on the impact of weight loss on the risk of cancer recurrence and mortality in breast cancer patients is limited so far.

Tirzepatide (Mounjaro, Lilly.S.A), also referred to as a 'twincretin', is a novel dual glucagon-like peptide-1 receptor agonist (GLP-1 RA) / glucose-dependent insulinotropic polypeptide (GIP). It is the first treatment designed as a single molecule to bind to both GIP and GLP-1 receptors that has emerged as effective pharmacologic agents for managing insulin resistance in type 2 diabetes but also for promoting sustained weight loss [8] Administered once weekly, tirzepatide offers dosing flexible dosing ranging from 2.5 mg to 15 mg per 0.5 mL injection. Preclinical data from the orthotopic E0771 mammary mouse model of obesity and triple-negative breast cancer treated with tirzepatide demonstrated substantial weight loss and attenuation of obesity-driven alterations in circulating metabolic hormone levels, together with a marked suppression of orthotopic E0771 tumor growth [9]. An additional study with GLP-1 revealed that this therapy also exhibits an anti-inflammatory effect through different mechanisms including the regulation of immune signaling pathways, which may prevent the release of inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) [10]. Thus, GLP-1RA encourage initiating anti-inflammatory mechanisms such as the AMP-activated protein kinase (AMPK) pathway, which helps suppress inflammation [11]. Furthermore, GLP-1RA has demonstrated the ability to reduce the activation of nuclear factor-kappa B (NF-κB), a key regulator of inflammation, leading to decreased production inflammatory mediators [10]. Furthermore, a post hoc analysis of a phase 2 trial found that treatment with Tirzepatide led to significant decreases in inflammatory biomarkers such as YKL-40, intercellular adhesion molecule 1 (ICAM-1), C-reactive protein (CRP), leptin, and growth differentiation factor 15 over 26 weeks [12].

Despite previous studies, there are currently no prospective data evaluating the role of novel GLP-1 receptor agonists in patients with breast cancer. Considering their effects on both weight loss and inflammation, we aim to investigate for the first time this crosstalk in a **prospective pilot study** involving patients with early triple-negative breast cancer (**TNBC**) receiving the KEYNOTE-522 regimen. The primary objective of this study is to assess the effect of **Tirzepatide** in combination with the **KEYNOTE-522 regimen** in TNBC patients with a **BMI >27**, compared with similar patients who do not receive GLP-1 therapy.

References:

1. Kim J, et al. Global patterns and trends in Breast Cancer incidence and mortality across 185 countries. Nat. Med. 2025,31, 1154–1162
2. Schmid P, Cortes J, et al, KEYNOTE-522 Investigators. Overall Survival with Pembrolizumab in Early-Stage Triple-Negative Breast Cancer. N Engl J Med. 2024 Nov 28;391(21):1981-1991.

3. World Health Organization. Obesity and Overweight; WHO: Geneva, Switzerland, 2025. Available online: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed on 18 May 2025).
4. Joo-Hui Han. Immuno-metabolic diseases and therapeutics: molecular mechanisms via inflammasome signaling. Cell Commun Signal. 2025 Aug 19;23:373.
5. Zhang X, et al. Obesity alters immunopathology in cancers and inflammatory diseases. Obes Rev. 2023;24(12): e13638.
6. Chan DS, et al. Body mass index and survival in women with breast cancer-systematic literature review and meta-analysis of 82 follow-up studies. Ann Oncol. 2014;25(10):1901–1914.
7. Litton JK, et al. Relationship between obesity and pathologic response to neoadjuvant chemotherapy among women with operable breast cancer. J. Clin. Oncol. 2008 26 (25), 4072–4077.
8. Brown KA, Simpson ER. Obesity and breast cancer: mechanisms and therapeutic implications. Front Biosci (Elite Ed). 2012 Jun 1;4(7):2515-24.
9. France NL, Syed YY. Tirzepatide: A Review in Type 2 Diabetes. Drugs. 2024 Feb;84(2):227-238.
10. Glenny EM, Ho AN, et al. Tirzepatide attenuates mammary tumor progression in diet-induced obese mice. bioRxiv [Preprint]. 2024 Jan 23:2024.01.20.576484.
11. Bendotti G, Montefusco L, Lunati ME, et al. The anti-inflammatory and immunological properties of GLP-1 receptor agonists. Pharmacol Res 2022; 182: 106320.
12. Krasner NM et al. Glucagon-like peptide-1 (GLP-1) analog liraglutide inhibits endothelial cell inflammation through a calcium and AMPK dependent mechanism. PLoS One 2014; 9: 1–11
13. J.M. Wilson, et al., The dual glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 receptor agonist tirzepatide improves cardiovascular risk biomarkers in patients with type 2 diabetes: a post hoc analysis, Diabetes Obes. Metab. 24 (1) (2022) 148–153.

Working hypothesis

The hypothesis of this study is to evaluate the anti-inflammatory and anti-tumour effect of Tirzerpatide in patients with a high BMI who are receiving neoadjuvant chemotherapy according to the KEYNOTE-522 regimen. The anti-inflammatory effect of GLP-1 associated with chemotherapy should be reflected not only in greater weight loss but also in other variables to be evaluated, such as breast MRI, ctDNA, and pathological response. Moreover, a comprehensive study will be carried out to identify prognostic and predictive markers in the maintenance of metabolic stress in patients with high BMI.

Material and Methods

The study consists of an **interventional clinical trial** combined with multidisciplinary approaches to better understand the role of obesity in the behavior of TNBC. To carry out this study, two TNBC cohorts will be prospectively included as follows (For more details please see **Figure 1 & 2**):

Cohort 1: 15 patients over 18 years of age with a diagnosis of initial triple-negative breast cancer, candidates to receive the KEYNOTE-522 regimen, and with a baseline BMI > 27. These patients will receive the GLP-1 agonist Tirzepatide (Munjaro, Lilly, S.A) for the duration of neoadjuvant chemotherapy (approximately 6 months, **Figure 2**).

Cohort 2: 15 patients over 18 years of age with a diagnosis of initial triple-negative breast cancer, candidates to receive the KEYNOTE-522 regimen, and with a baseline BMI > 27 (**Figure 2**).

Triple negative breast cancer will be defined as a tumor with ER, PR expression less or equal to 9% and HER2 negative. Evaluation of TILs (tumor infiltrated lymphocytes) will be also evaluated at baseline biopsy. All patients will undergo baseline breast MRI (T1), intermediate MRI (between CBP-Taxol and anthracyclines) (T2), and pre-surgery MRI (T3). Patients will also be evaluated with a ctDNA liquid biopsy (Signatera) at T1, T2, and T3. Once neoadjuvant treatment is complete, patients will undergo surgery to assess pathological response. Leptin and adiponectin blood levels will be assessed at T1and T3 points in both cohorts.

Additionally, from a **translational research perspective**, immunohistochemical analyses will be performed to characterize the metabolic and immune components of tumors at baseline. These analyses will assess FASN, ROS, and mTOR/AKT/PI3K pathway activation; inflammatory cytokines; and tumor-infiltrating immune cells such as $CD8^+$ T cells, $CD4^+$ T cells, and NK cells. In addition, pyroptosis markers, including NLRP1/3 and Gasdermin family proteins—the final executors of pyroptosis—will be evaluated. Tissue samples collected during the study will be examined, and the expression of each biomarker will be correlated with various clinicopathological features obtained throughout the study.

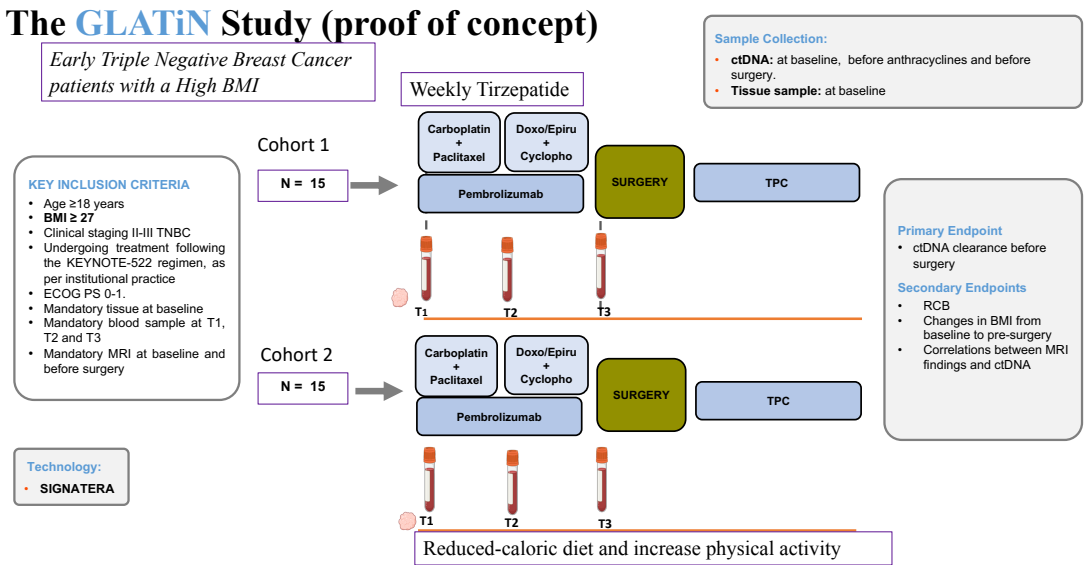

Figure 1. Schematic representation of the G_LATIN study (proof of concept). It has been summarized the enrollment of TNBC patients, the treatment regimen, and the biological samples that will be collected to evaluate both therapeutic and biological responses.

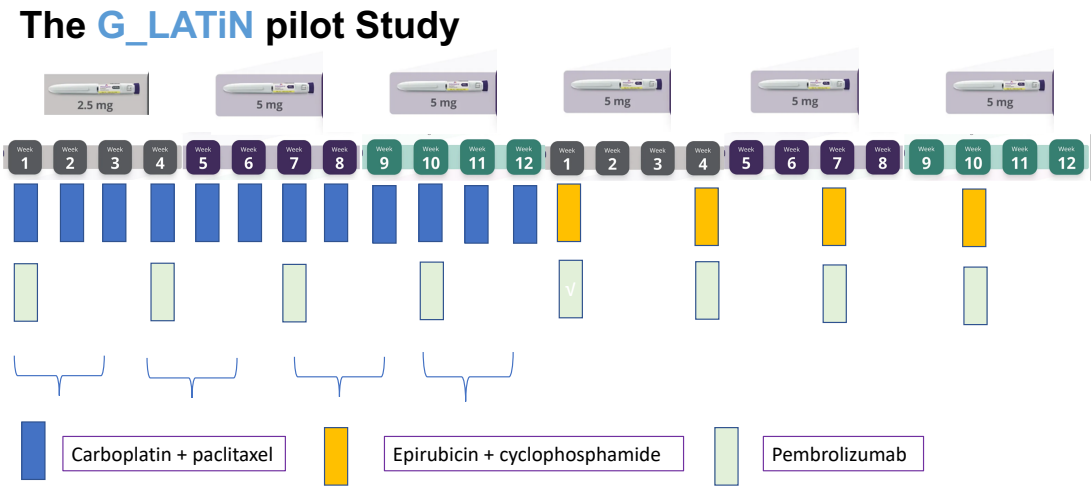

Figure 2. Schematic representation of the G_LATIN treatment protocol in TNBC patients with a BMI >27. The figure illustrates the study design, including the immunotherapy and chemotherapy components following the KEYNOTE-522 regimen, as well as the GLP-1 receptor agonist treatment cycles administered during the GLATIN study. Basically, five doses of Tirzepatide will be administered (one 2.5 mg and the following four 5 mg). Time (numbers) in weeks.

Millestones:

Clinical Objectives: **(1)** Assess weight loss in each cohort at time point T3 prior to surgery. **(2)** Evaluate ctDNA clearance at different time points T2 and T3 prior to surgery between one cohort and another. **(3)** Correlate MRI findings with ctDNA at T2 and T3 in both cohorts. **(4)** Assess pCR in both cohorts. **(5)** Safety of the combination (Tirzepatide and KEYNOTE-522 regimen)

Translational Objectives: **(1)** Identify predictive biomarkers (e.g., FASN, GSDM, mTOR/AKT/PI3K activation, cytokine profiles, pyroptosis markers) that could help select patients most likely to benefit from GLP-1–based therapies. **(2)** Improve therapeutic strategies by determining whether metabolic modulation can increase the therapeutic response **(3)** Advance personalized medicine approaches for TNBC patients with obesity or ober-weigth by linking metabolic status, immune response, and clinical outcomes.

Schedule and budget:

The project will be carried out over two years and, in order to reach 30 patients, at least five centers should participate, including at least six patients per year.

BUDGET	**EUROS**
GLP-1 (Munjaro)	23.450
Signatera test (4000 euros/patient)	120.000
Translational research (immunohistochemistry, cytometry, marker testing in blood and tissue, Leptin and adiponectin)	15.000
Publications	8.000
Statistical Analysis	2.000
Subtotal	168.450
Indirect cost (20%)	33.690
TOTAL	**202.140**