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# Obesity and overweight in R/R DLBCL patients is associated with a better response to treatment of R2-GDP-GOTEL trial. Potential role of NK CD8 + cells and vitamin D

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## Abstract

**Background** Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin's lymphoma worldwide and is characterized by its heterogeneity. Although first-line therapy improves survival outcomes for DLBCL patients, approximately one third will relapse, often with a poor prognosis. Among the factors influencing prognosis and response to treatment in cancer patients, including those with lymphoma, overweight and obesity have emerged as significant considerations. However, the role of excess weight in DLBCL remains controversial, with studies reporting both negative and positive effects on cancer outcomes. In this translational substudy of the R2-GDP-GOTEL trial, we have evaluated the impact of excess weight as a predictor of treatment response and survival in patients with relapsed/refractory (R/R) DLBCL, and examining its relationship with immune cell dynamics.

**Methods** Of the 79 patients who received the R2-GDP scheme in the phase II trial, weight and height parameters were obtained in 75 patients before starting treatment. Blood samples were analyzed by flow cytometry. Statistical analyses were performed to determine the prognostic value of overweight and obesity at baseline in R/R DLBCL patients.

**Results** Our results indicate that overweight (including obese) patients exhibit longer survival compared to patients of ideal weight. This group also demonstrated a reduction of regulatory T cells with supposedly protumor activity and an increase of Natural Killer (NK)-like T cells with supposedly antitumor activity. Additionally, we have

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found that excess weight correlates with better treatment response, associated with elevated levels of vitamin D and CD8 + NK cells.

**Conclusions** Our findings suggest that excess weight does not exacerbate the progression of DLBCL. Instead, it appears to confer a survival advantage and improve treatment response, with the immune system playing a possible pivotal role in mediating these effects.

**Trial registration** EudraCT, ID:2014–001620-29.

**Keywords** Diffuse Large B Cell Lymphoma, Response to treatment, Obesity paradox, CD8 + Natural Killer cells, Vitamin D, R2-GDP-GOTEL

## Background

Diffuse large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma worldwide, accounting for approximately 40% [1]. This tumor is characterized by its clinical heterogeneity, with variations in presentation, response to therapy, and prognosis. In recent years, first-line therapies, particularly those involving rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone (R–CHOP), have led to significant improvements in survival outcomes for DLBCL patients. Despite these advances, about one-third of patients experience relapse and face poor prognoses, especially in cases where the disease is refractory to initial or subsequent treatments [1, 2]. Due to the heterogeneity of diffuse large B-cell lymphoma (DLBCL), researchers face the challenge of identifying new prognostic factors that can predict treatment response in patients with DLBCL. Among these factors, excess weight has emerged as a significant consideration across various tumor types, including lymphomas. Excess body weight, defined as an abnormal or excessive accumulation of fat, encompasses both overweight and obesity.

It is well-established that obesity increases the risk of developing multiple malignancies. In the context of non-Hodgkin lymphomas, particularly DLBCL, obesity has traditionally been viewed as a risk factor [3, 4]. Moreover, excess weight is known to worsen prognosis and may influence treatment decisions in patients with DLBCL [5]. A meta-analysis that pooled 203 studies involving over 6 million cancer patients demonstrated that obesity is associated with reduced overall survival in this population [6]. However, in this and other studies it was observed that patients with melanoma, lung and kidney cancer [6], breast [7] or head and neck cancer [8, 9], exhibited better survival rates if they were obese. This inverse association is being called "obesity paradox" [10] and the underlying mechanisms by which obesity might serve as a protective factor for certain cancer types remain unknown.

Regarding DLBCL, the limited number of previous studies present mixed results. Some studies have shown that DLCBL patients who were obese prior to diagnosis had a poorer survival outcome [11]. Conversely, being overweight or obese at the time of DLBCL diagnosis has been associated with improved overall survival [12, 13]. While some research has identified a relationship between excess weight and survival in lymphoma patients, it remains unclear whether obesity can influence the treatment response in these individuals. One explanation could imply that excess weight patients present differences in immune cell composition compared to those with ideal weight, as the immune system plays a crucial role in the prognosis and treatment response of DLBCL patients [1]. Indeed, our previous results showed that promising and underexplored subsets of NKs, CD8 + NKs, is associated with longer survival and complete responses to treatment in R/R DLBCL patients [1].

In patients with DLBCL enrolled in the GOTEL clinical trial, our group has demonstrated that overweight individuals exhibit better survival outcomes compared to those with an ideal weight. Moreover, a higher proportion of overweight patients achieve a complete response to treatment. To further investigate the underlying reasons for these differences in response to treatment, we conducted an immunological profiling of the patients. Our analysis revealed significant differences in the CD8 + Natural Killer (NK) cell population, which has been previously associated with complete response and survival in this treatment regimen [1]. Notably, we observed these differences between patients with ideal weight and those with excess weight. Additionally, for the first time, we describe a relationship between treatment in excess weight patients, CD8 + NK cell population and vitamin D levels.

## Methods

### Patients and ethics approval

A total of 79 patients with R/R DLBCL participated in the R2-GDP-GOTEL phase II clinical trial (EudraCT Number: 2014–001620-29) [14]. This was a multicenter, open-label, single-arm study. The research was conducted according to the International Ethical Guidelines

for Biomedical Research Involving Human Subjects, the Declaration of Helsinki, good clinical practice guidelines, and local laws. The study protocol, along with any subsequent amendments, received approval from the Seville Provincial Ethics Committee for Research with Drugs.

### Body mass index: patients characteristics

Prior to the initiation of treatment, weight and height measurements were collected from trial participants. Body mass index (BMI) was calculated by dividing weight (kg) by height (m) squared (kg/m$^2$) for each patient. Patients were classified according to their BMI based on the World Health Organization (WHO) as underweight (BMI < 18.5 kg/m$^2$), ideal weight (BMI 18.5–24.9 kg/m$^2$), overweight (BMI 25–29.9 kg/m$^2$), and obesity (BMI ≥ 30 kg/m$^2$) [15]. Of the 79 patients in the study, three patients were discarded because two of them had no height or weight data and the other was rule out because the patient had a BMI < 18.5 kg/m$^2$. Therefore, the cohort of cases analyzed consisted of 75 patients whose main characteristics are summarized in Supplementary Table 1.

### Treatment of patients: Lenalidomide plus R-GDP (R2-GDP)

Patients with relapsed/refractory (R/R) DLBCL who were not candidates for autologous stem cell transplantation (ASCT) received treatment with a combination of lenalidomide and rituximab (R2) along with gemcitabine, cisplatin, and dexamethasone (R2-GDP schedule). The administration protocol has been previously detailed [1, 14].

### Response to treatment of patients

Tumor response was assessed using the International Working Group Criteria [16]. Computed tomography scan was performed after the third induction cycle, and PET scans were conducted within the four weeks following the final induction cycle. The Best Overall Response was used to calculate the response. This evaluation allowed us to categorize patients into complete response (CR), partial response (PR), stable disease (SD) or and those with disease progression (PD).

### Immunophenotyping in whole blood samples

Immune cells were studied in peripheral blood from R/R DLBCL patients during the R2-GDP-GOTEL study at baseline time-point. Blood samples were collected in EDTA-K3 tubes and cell populations were determined by flow cytometry analysis using the BD FACSCanto II™ flow cytometry system with the monoclonal antibodies (mAbs) and protocols recommended by Becton Dickinson Immunocytometry Systems (BDIS, San Jose, CA, USA). Lymphocyte subpopulations were analyzed by BD Muttitest 6-Color TBNK (Becton Dickinson). mAbs are listed in Supplementary Table 2 and the phenotypes for immune cell studies are described in Supplementary Table 3.

### Vitamin D analysis in serum

Vitamin D levels could only be analyzed in 62 R/R DLBCL patients before treatment. Blood from patients was collected in serum separator tubes and after 30 min at rest was centrifuged at 2000 rpm 10 min and serum on top of the gel was collected in cryotubes and stored at −80 °C. At the end of the R2-GDP-GOTEL study, all samples were measured in the same day by an automated chemiluminescence immunometric analysis using the Liaison® (DiaSorin, Madrid, Spain) system.

### Statistical analysis

Mann–Whitney tests was used to evaluate differences between two groups. Survival curves were plotted using the Kaplan–Meier method and compared with the log-rank test. The Spearman's Rank test and principal component analysis were used to determine the relationship between different variables. Fisher exact test was used to assess the association between two binary variables in a contingency table. All statistical analyses in the study were performed using the software GraphPad Prism (6.01) and JMP (V.10). The average of samples with SD is presented in all experiments. For all analyses, $p$-values of ≤ 0.05 were considered statistically significant.

## Results

### Baseline clinical characteristics of R/R DLBCL patients according to BMI

A total of 75 patients diagnosed of R/R DLBCL enrolled in the R2-GDP-GOTEL trial were categorized by BMI into two groups based on their BMI: ideal weight (BMI 18.5–24.9 kg/m$^2$) and excess weight (BMI ≥ 25 kg/m$^2$). The ideal weight group consisted of 25 patients (median age of 63 years) and the excess weight group included 50 patients (median age of 68.6 years). In the ideal weight group, 60% were male and 40% were female and similarly in the excess weight group, 52% were male and 48% were female. Regarding disease status, 44% of the ideal weight group were primary refractory DLBCL defined as in the SCHOLAR-1 study [17] and 52% had relapsed non-refractory disease. For the excess weight group, 38% had refractory disease and 52% had relapsed non-refractory disease. The cell-of-origin subgroups (Hans algorithm by immunohistochemistry) showed that 24% of the ideal

weight group were germinal center B-cell (GCB), and 44% were non-GCB, while in the excess weight group, 26% were GCB, and 46% were non-GCB. In terms of treatment response, 32% of the ideal weight group achieved a CR and 40% had PD. In the excess weight group, 42% achieved a CR and 26% had PD. The main baseline characteristics of the patients are summarized in Supplementary Table 1.

## Survival and response to treatment does not vary between overweight and obese patients

We initially investigated whether survival outcomes differed between overweight and obese patients with R/R DLBCL. Our analysis revealed no significant differences in survival between these two groups at 24 months ($p=0{,}9308$) or at the conclusion of the study ($p=0{,}7599$) (Fig. 1A and B). Additionally, no changes were found between alive and deceased overweight and obese patients with OS greater than and less than 24 months ($p=0.6684$), nor at the end of the study ($p=0.8848$) (Fig. 1C and D). Finally, we also examined the response to treatment between these two groups of patients with R/R DLBCL. There was no difference in treatment response between these groups of patients ($p=0.1088$) (Fig. 1E).

## Overweight/Obese R/R DLBCL patients exhibit longer survival compared to Ideal weight Patients

Given that no significant differences in survival were found between overweight and obese patients, these two categories were combined into a single group referred to as "excess weight patients" characterized by a BMI $\geq 25$ kg/m$^2$. For the remainder of the study, comparisons were made between ideal weight patients and those with excess weight.

Our analysis revealed that patients with excess weight demonstrated a significantly higher survival rate compared to those with ideal weight, both at 24 months ($p=0.0413$) and at the study's conclusion ($p=0.0352$) (Fig. 2A and B). Additionally, the proportion of patients with excess weight who were alive at 24 months (43%) was statistically greater than that of patients with ideal weight (28%) ($p=0.0382$) (Fig. 2C). A similar trend was observed at the end of the treatment period ($p=0.0002$), where 39% of patients with excess weight were still alive, compared to just 15% of patients with ideal weight (Fig. 2D). To rule out that the result obtained could be influenced by confounding factors, we performed an analysis with the clinical characteristics of patients with excess weight. No association was observed with age, sex, molecular subtypes, and refractory disease (Supplementary

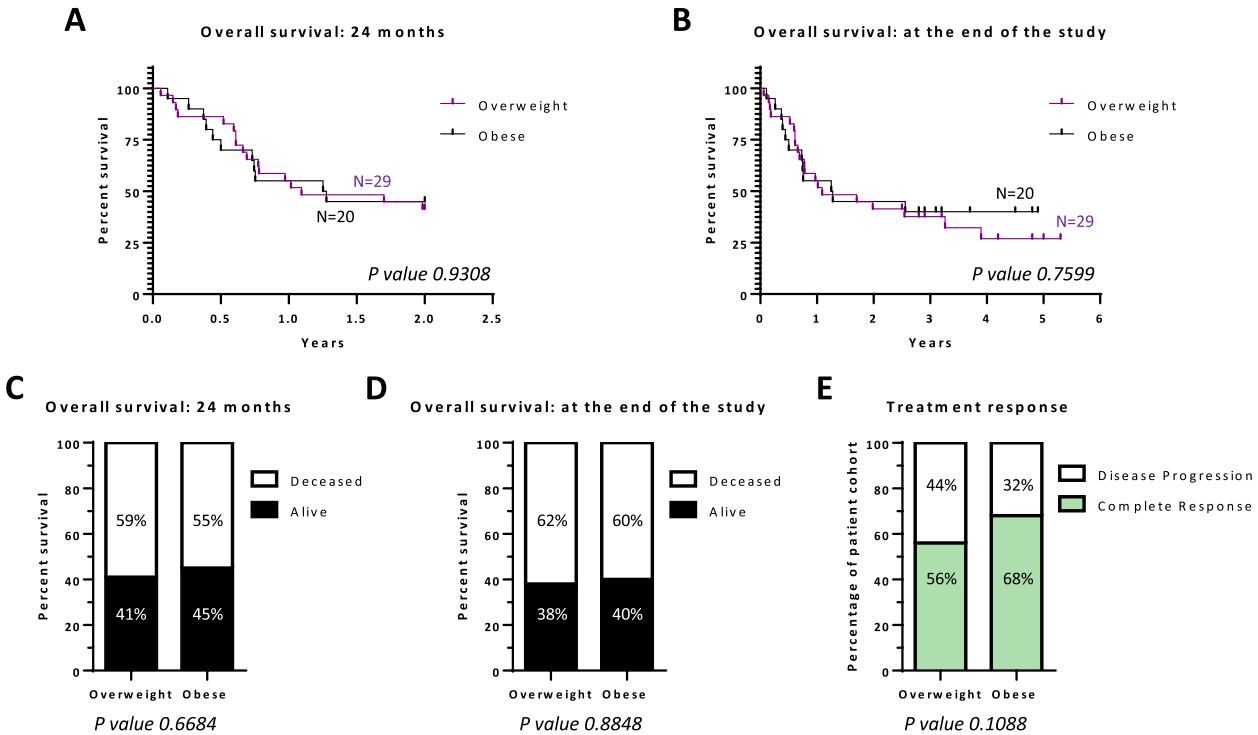



**Fig.1** Survival outcome does not vary between overweight and obese patients. Study of overall survival between obese and overweight patients at 24 months (**A**) and at the end of the study (**B**) using Kaplan–Meier curves. Percentage of patients alive at 24 months after treatment (**C**) and at the end to the study (**D**). Analysis of the percentage of patients according to treatment response (**E**)

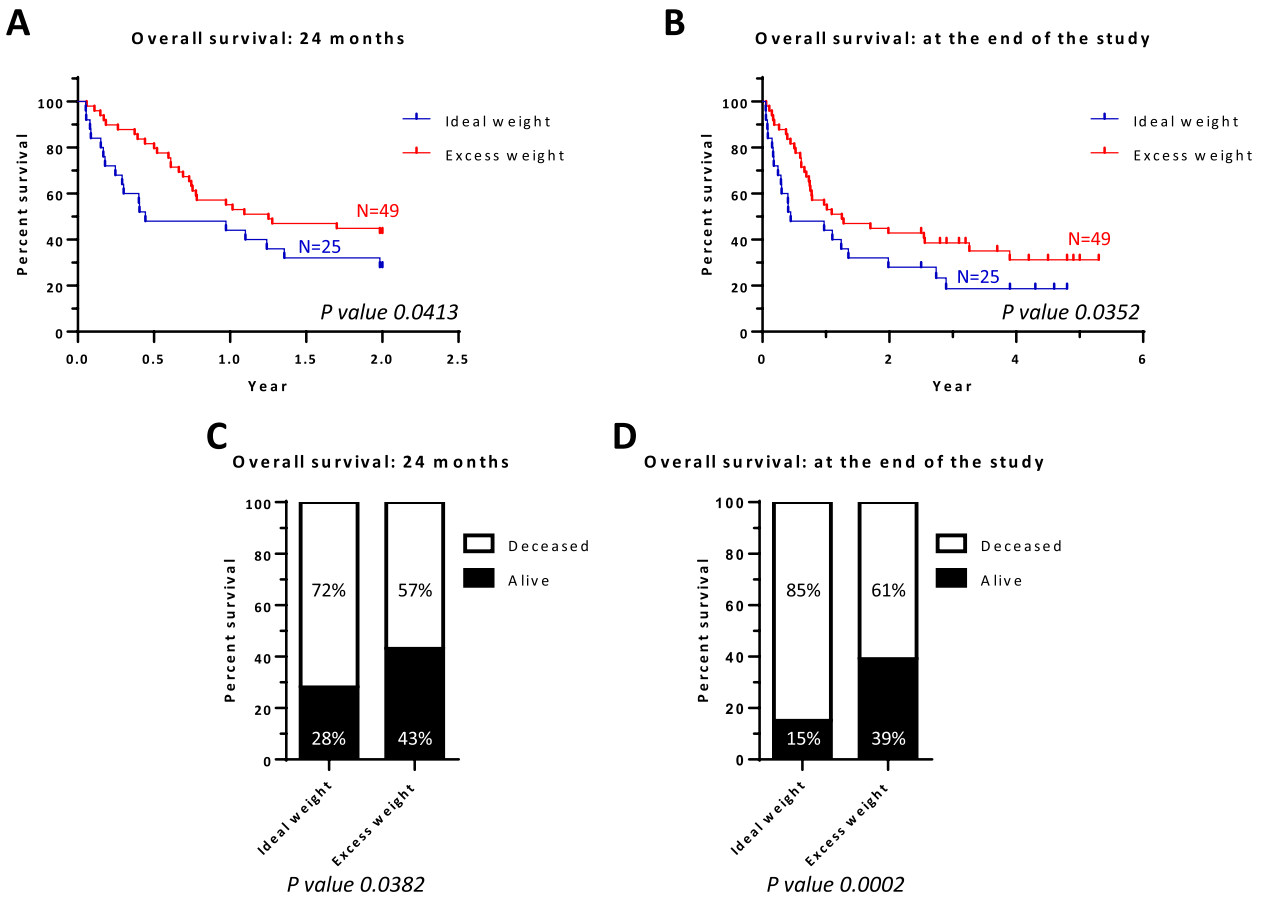



**Fig. 2** Excess weight patients have longer survival compared to ideal weight patients. Kaplan–Meier curves of overall survival between ideal-weight and excess weight patients at 24 months (**A**) and at the end of the study (**B**)**.** Percentage of patients alive at 24 months after treatment (**C**) or at the end to the study (**D**)

Fig. 1A). These findings suggest that excess weight may act as a predictive factor in R/R DLBCL patients treated with the R-GDP and lenalidomide schedule, as evidenced by their improved survival outcomes relative to those with ideal weight.

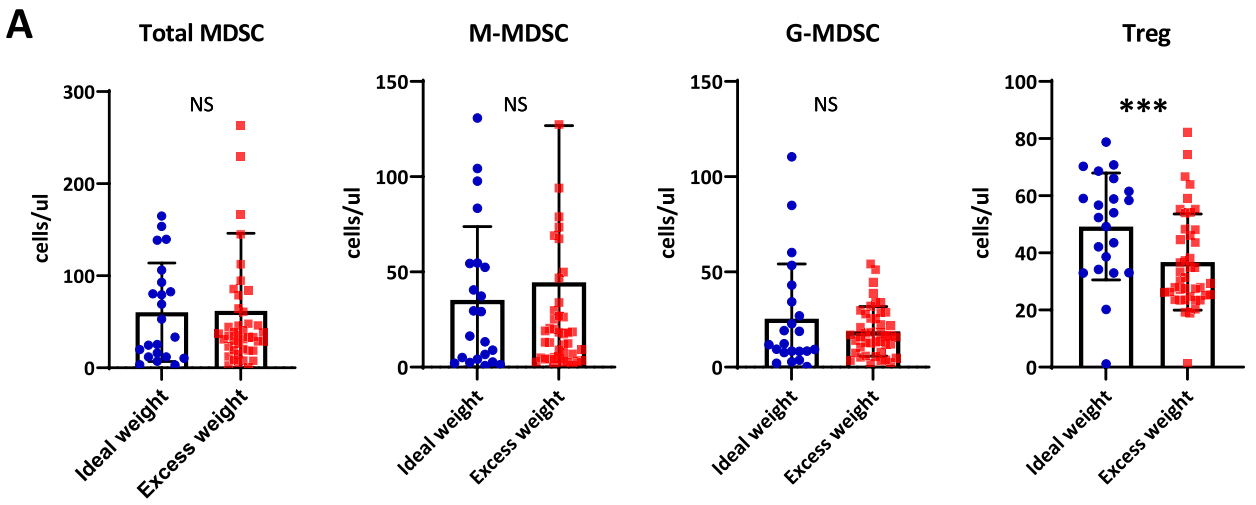
## Reduced circulating regulatory T cell (Treg) population in patients with excess weight

To investigate the underlying reasons for the improved survival observed in patients with excess weight, we conducted an immunological profiling analysis focusing on pro-tumor and anti-tumor immune cell populations in both excess weight and ideal weight patients.

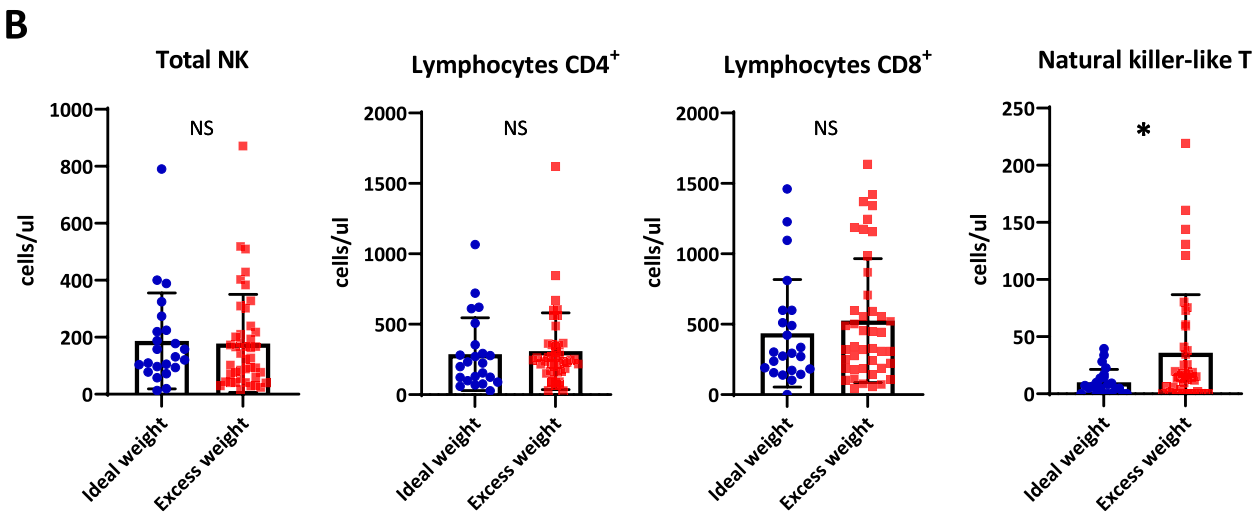
Regarding pro-tumor immune cells, we found no significant differences between the two groups in the subsets of myeloid-derived suppressor cells (MDSCs), including monocytic-MDSCs (M-MDSCs), granulocytic-MDSCs (G-MDSCs), and total MDSCs ($p=0.9179$ for M-MDSCs, $p=0.9069$ for G-MDSCs, and $p=0.9069$ for total MDSCs) (Fig. 3A). However, a significant reduction in the regulatory T cell (Treg) population was observed in patients with excess weight compared to those with ideal weight ($p=0.0042$) (Fig. 3A). Among the anti-tumor immune cells analyzed, we found significant differences in NK-like T cells (CD3 + CD8 + CD16 + CD56 +), a cytotoxic T cell subset known to induce tumor cell death. Specifically, NK-like T cells were increased in the peripheral blood of excess weight patients ($p=0.0306$) (Fig. 3B). No significant differences were observed in the other anti-tumor cell populations (Fig. 3B).

Finally, we evaluated the relationship of Treg and NK-like T cells populations with survival in obese patients. We observed that overall survival in these patients is positively related to NK-like T cells and negatively related to Treg cells (Supplementary Fig. 1B). However, this relationship is not significant (Supplementary Fig. 1C).

In summary, patients with excess weight exhibit a decreased population of pro-tumor Treg cells and an increased population of anti-tumor NK-like T cells, which may contribute to their improved outcomes.




| Pro-tumor immune cells | Ideal weight | Excess weight | p value |
|---|---|---|---|
| Total MDSCs | 60,3 (3,1-164,9) | 61,9 (0,8-463,9) | 0,9069 |
| M-MDSC | 35,4 (0,8-130,8) | 44,6 (0,3-450,7) | 0,9179 |
| G-MDSC | 25,4 (0,3-110,4) | 18,8 (1,9-54,2) | 0,9069 |
| Treg | 49,2 (1,1-78,8) | 36,8 (1,2-82,3) | 0,0042 |




| Anti-tumor immune cells | Ideal weight | Excess weight | p value |
|---|---|---|---|
| NK-like T cells | 10 (0-39,5) | 35,8 (0-219,2) | 0,0306 |
| Lymphocytes CD8+ | 435,7 (0-1461,5) | 526,3 (42,5-1638) | 0,4621 |
| Lymphocytes CD4+ | 287,3 (27,6-1066,5) | 308,7 (28,8-1618,5) | 0,5588 |
| Total NK | 187,2 (13,2-790) | 178,2 (17,2-871,5) | 0,5769 |

**Fig. 3** Specific immune cells subpopulations between ideal and excess weight patients. Analysis of basal levels of pro-tumor immune cells among ideal weight and excess weight patients (**A**). Analysis of basal levels of anti-tumor immune cells among ideal weight and excess weight patients (**B**). For all the analyses, **P* ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 and ****P ≤ 0.0001. NS, not significant

## Excess weight in R/R DLBCL patients is associated with improved treatment response in GOTEL trial

We next assessed treatment response in the excess weight and ideal weight patient groups, focusing on those who achieved complete response (CR) versus those with disease progression (PD). A significantly higher percentage

of CR was observed in patients with excess weight (60%) compared to those with ideal weight (44%) ($p=0.0335$) (Fig. 4A).

To explore the immune mechanisms underlying this enhanced response to treatment, we analyzed the baseline profiles of pro- and anti-tumor immune cell populations in both patient groups. Regarding pro-tumor cells, no significant differences were identified between CR and PD in either ideal weight or excess weight patients (Fig. 4B). Similarly, T lymphocyte levels did not differ between these groups (Fig. 4B). However, in the ideal weight group, an increase in NK-like T cells was observed in patients who achieved CR compared to those with PD ($p=0.0151$) (Fig. 4B). Interestingly, this trend was not significant in the excess weight group (Fig. 4B).

Further analysis of anti-tumor cells revealed differences only in the excess weight group, where patients who achieved CR had significantly higher levels of total NK cells ($p=0.0108$) (Fig. 4B).

Given our previous findings that the CD8 + NK cell subpopulation is associated with CR in R/R DLBCL patients [1], we further investigated this subpopulation in both excess weight and ideal weight groups. Remarkably, the group of excess weight patients with CR had significantly higher baseline levels of CD8 + NK cells compared to those with PD ($p=0.0036$) (Fig. 4B). No significant differences in treatment response were observed in the CD8- NK subpopulation in either group (Fig. 4B).

In summary, our results suggest that excess weight is associated with better treatment response in R/R DLBCL patients, and elevated circulating CD8 + NK cell levels at baseline are strongly linked to CR in this patient population.

### High levels of vitamin D and CD8 + NK cells are associated with CR to treatment in excess weight in R/R DLBCL patients

Given that vitamin D may influence treatment efficacy in cancer patients [18], we first determined whether baseline vitamin D levels were related to treatment response. It was observed that there is no difference in response to treatment in ideal weight patients between those who are vitamin D deficient (< 15 ng/mL) and those who have sufficient vitamin D levels (> 15 ng/mL) (Fig. 5A). However, the percentage of patients with excess weight and CR to treatment presented higher vitamin D levels than those with PD (44% and 22% respectively) (Fig. 5B). This relationship between vitamin D and response to treatment is not influenced by excess weight, since we have found that patients with ideal weight have the same vitamin D levels as patients with excess weight (Supplementary Fig. 1D).

Considering the observed relationship between vitamin D and treatment response, we next investigated the correlation of vitamin D with CD8 + NK cells. Our analysis revealed a positive correlation between vitamin D levels and CD8 + NK cells in the excess weight group ($p=0.0474$) but not in the ideal weight group ($p=0.8167$) (Fig. 5C and D). Furthermore, patients with sufficient vitamin D levels (> 15 ng/mL) exhibited significantly higher NK CD8 + cell counts than those with vitamin D deficiency (< 15 ng/mL) ($p=0.0316$) (Fig. 5E). This relationship was not observed for the NK CD8- or total NK cell populations ($p=0.1873$ and $p=0.5673$, respectively) (Fig. 5E).

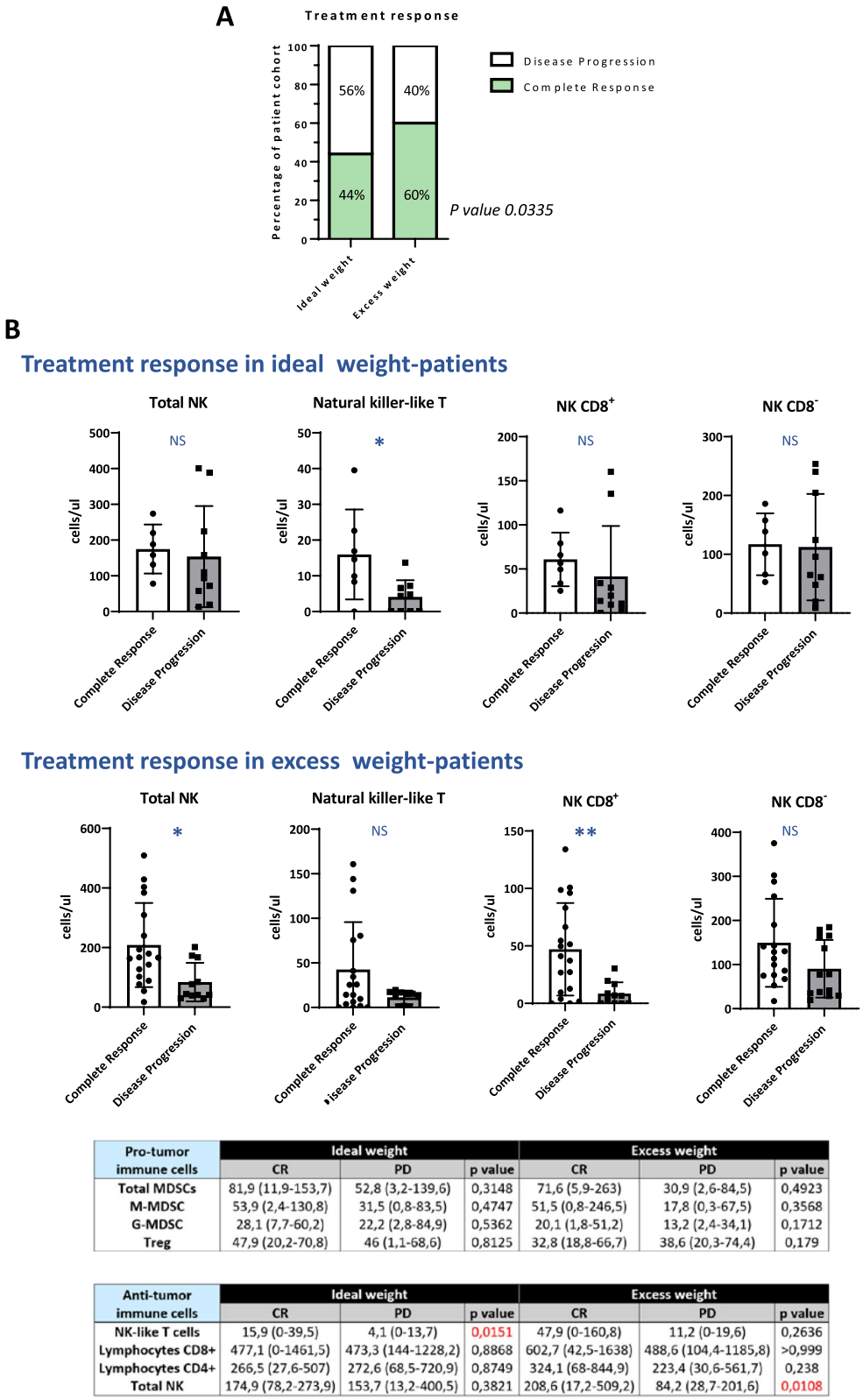
These findings suggest that, in patients with excess weight, baseline vitamin D levels are positively correlated with CD8 + NK cell counts, and this relationship may contribute to an improved response to treatment in R/R DLBCL patients.

## Discussion

Obesity is recognized as a risk factor for the development and mortality of numerous diseases, including cancer. Specifically, obesity has been associated with an increased risk of developing DLCBL and may also contribute to a poorer prognosis in these patients [4, 19]. However, the impact of excess weight on survival outcomes in DLBCL patients remains. In our study we did not observe any significant differences in survival between overweight and obese patients, a finding consistent with previous reports in patients with colon cancer [20] and lung cancer [21].

Numerous studies have shown that cancer patients who are obese or overweight often experience lower survival rates, establishing obesity as an adverse prognostic factor. For instance, Geyer et al. demonstrated that patients with DLBCL who were obese prior to diagnosis had worse survival outcome [11]. However, there are also several studies indicating an inverse relationship, where being overweight or obese at the time of DLBCL diagnosis is associated with improved overall survival [12]. These findings support the results presented in our study, where patients with R/R DLBCL and excess weight (BMI > 25 kg/m2) at baseline have better survival outcome. Furthermore, the percentage of overweight patients alive at 2 years and at the conclusion of the study was higher compared to those with an ideal weight.

The reasons why obesity could serve as a protective factor in cancer patients remain unclear. One possible explanation is that excess adipose tissue in cancer patients may provide a reserve of energy that helps patients endure the disease and withstand therapy [22]. In contrast, malnutrition and underweight conditions may compromise immune function increasing susceptibility to infections, treatment-related toxicity, and metastasis [8, 9]. Another plausible explanation involves differences in




| Pro-tumor immune cells | Ideal weight | | | Excess weight | | |
|---|---|---|---|---|---|---|
| | CR | PD | p value | CR | PD | p value |
| Total MDSCs | 81,9 (11,9-153,7) | 52,8 (3,2-139,6) | 0,3148 | 71,6 (5,9-263) | 30,9 (2,6-84,5) | 0,4923 |
| M-MDSC | 53,9 (2,4-130,8) | 31,5 (0,8-83,5) | 0,4747 | 51,5 (0,8-246,5) | 17,8 (0,3-67,5) | 0,3568 |
| G-MDSC | 28,1 (7,7-60,2) | 22,2 (2,8-84,9) | 0,5362 | 20,1 (1,8-51,2) | 13,2 (2,4-34,1) | 0,1712 |
| Treg | 47,9 (20,2-70,8) | 46 (1,1-68,6) | 0,8125 | 32,8 (18,8-66,7) | 38,6 (20,3-74,4) | 0,179 |

| Anti-tumor immune cells | Ideal weight | | | Excess weight | | |
|---|---|---|---|---|---|---|
| | CR | PD | p value | CR | PD | p value |
| NK-like T cells | 15,9 (0-39,5) | 4,1 (0-13,7) | 0,0151 | 47,9 (0-160,8) | 11,2 (0-19,6) | 0,2636 |
| Lymphocytes CD8+ | 477,1 (0-1461,5) | 473,3 (144-1228,2) | 0,8868 | 602,7 (42,5-1638) | 488,6 (104,4-1185,8) | >0,999 |
| Lymphocytes CD4+ | 266,5 (27,6-507) | 272,6 (68,5-720,9) | 0,8749 | 324,1 (68-844,9) | 223,4 (30,6-561,7) | 0,238 |
| Total NK | 174,9 (78,2-273,9) | 153,7 (13,2-400,5) | 0,3821 | 208,6 (17,2-509,2) | 84,2 (28,7-201,6) | 0,0108 |

**Fig. 4** The response to treatment is more favorable in excess weight patients than in ideal weight. Analysis of the percentage of ideal weight and excess weight patients according to treatment response (**A**). Study of basal levels of immune cells in ideal weight (**B**) and overweight (**C**) patients in relation to response to therapy.For all the analyses, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ and $^{****}P \leq 0.0001$. NS, not significant

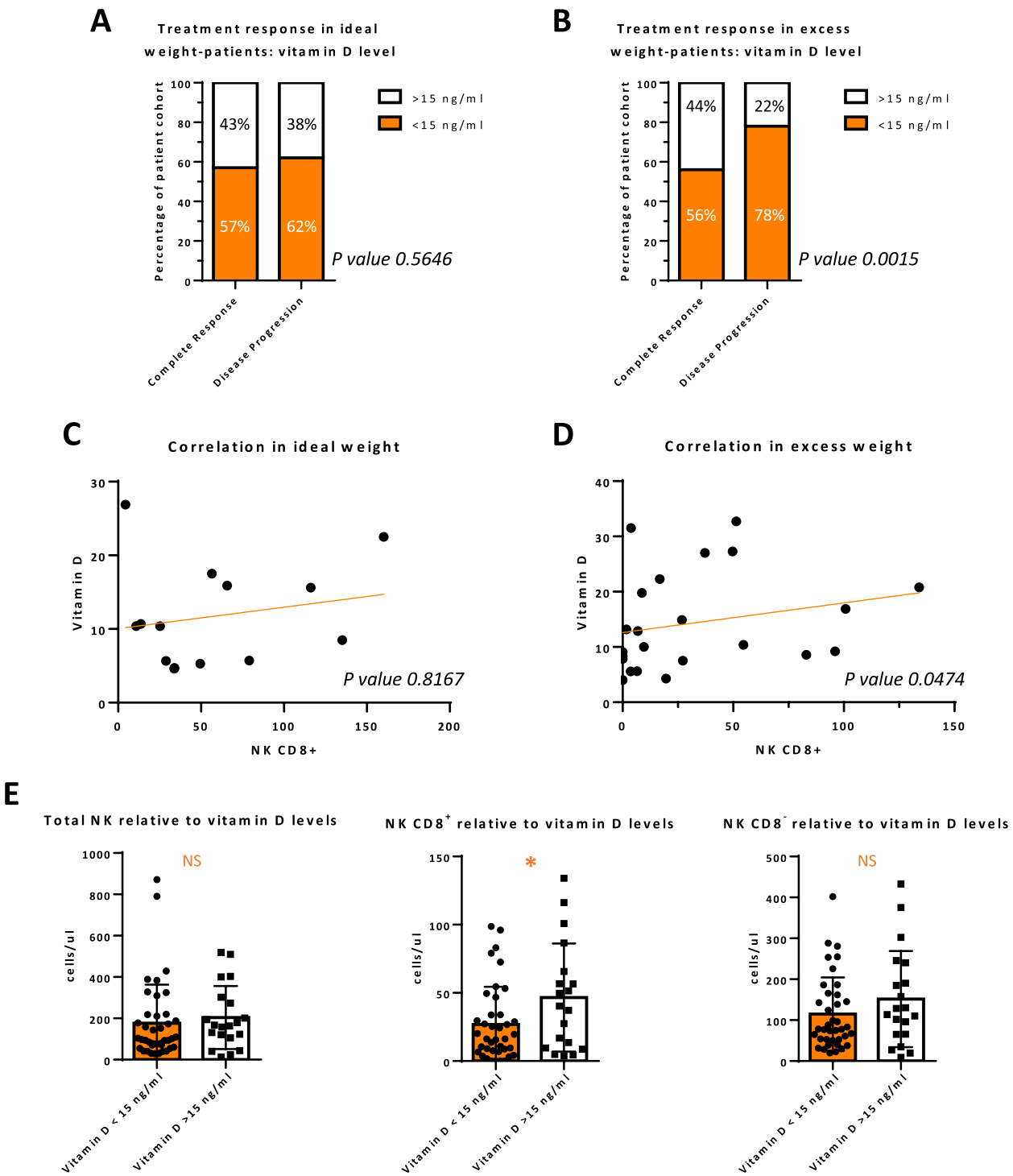



**Fig. 5** High levels of vitamin D is associated with CR to treatment and NK CD8 + population in excess weight patients. Relationship of vitamin D levels to treatment response in ideal weight (**A**) and overweight patients (**B**). Correlation between basal vitamin D levels and circulating NK CD8 + cells in ideal weight (**C**) and overweight (**D**) patients. Analysis of basal levels of total NK cells and $CD8^+$ and $CD8^-$ subpopulations according to vitamin D levels (< and > 15 ng/mL) (**E**). For all the analyses, $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ and $^{****}P \leq 0.0001$. NS, not significant

the composition of pro- and anti-tumor immune cells, given that the immune system plays a crucial role in DLBCL prognosis and treatment response [1, 23]. In our study, patients with excess weight showed increased levels of NK-like T cells, which may contribute to an effective anti-tumor immune response due to their cytotoxic capacity. Notably, elevated levels of NK-like T cells have been associated with improved survival in patients with chronic lymphocytic leukemia [24] and decreased levels of tumor infiltrating NK-like T cells are associated with tumor progression and poor survival in patients with gastric cancer [25]. In addition, these patients also exhibit decreased levels of Treg cells, which have been reported to mediate immunosuppression in patients with DLBCL [23]. In this regard, it has been reported that circulating Treg cell levels are reduced in obese patients regardless of cancer status [26]. Moreover, there is a reported association between circulating Treg cells and measures of adiposity, inflammation and glucose intolerance [27].

In addition to the "obesity paradox" observed in cancer patient survival, it has also been shown that obesity can influence treatment efficacy. On the one hand, several studies have reported that breast cancer patients with a high BMI are less likely to achieve a complete pathologic response after neoadjuvant chemotherapy [28, 29]. On the other hand, obesity appears to have a positive impact on treatment outcomes in cancer patients receiving immunotherapy. For instance, better responses have been observed in metastatic melanoma patients treated with ipilimumab [30], as well as in patients with melanoma, renal cancer, or lung cancer treated with immune checkpoint inhibitors [31]. The results in our study align with this trend, showing improved treatment responses in excess weight patients compared to those with normal weight. However, prior studies focusing on DLBCL have reported that BMI does not significantly impact treatment response in patients receiving R-CHOP therapy [32]. This discrepancy could be explained by the fact that previous studies did not group overweight and obese patients together as we did in our study, and that differences in treatment regimens may also account for this variation. In this line, the immunomodulatory effects of lenalinomide used in this study could partially account for the better response to treatment in the group of excess weight.

Surprisingly, obese patients who achieved CR exhibited higher levels of circulating CD8 + NK cells. Previously, our group already described that elevated baseline levels of circulating CD8 + NK cells were associated with a CR to treatment in R/R DLBCL patients [1]. This NK cell subtype is known for its potent cytotoxic activity [33–37] and has been linked to slower disease progression in several conditions [38]. NK cells present express various surface markers, including vitamin D receptors, which are crucial for their proper development [39]. It has been reported that vitamin D plays an essential role in supporting NK cell function [40, 41]. In aggressive B-cell lymphoma, vitamin D is known to have effects on the anti-tumor response promoted by the immune system [42], and its supplementation causes an improvement in the cytotoxicity of NK cells [43]. In addition, Vitamin D deficiency has been consistently identified as a negative prognostic factor in B-cell lymphomas [44–47], including those treated with rituximab and lenalidomide [48, 49]. Supplementing vitamin D has been shown to enhance the cytotoxicity mediated by these drugs in hematological malignancies [50, 51]. Moreover, sufficient vitamin D levels have been linked to improved treatment outcomes in cancer patients [18]. This evidence supports the novel findings in our study, which, for the first time, describe a relationship between baseline vitamin D levels and CD8 + NK cells in predicting treatment response among overweight patients with DLBCL. Specifically, vitamin D levels greater than 15 ng/mL were associated with a better treatment response in these patients.

In conclusion, excess weight may confer a survival advantage and enhance treatment response in R/R DLBCL patients treated with a combination schedule that includes the immunomodulatory drug lenalidomide. This protective effect could be partially attributed to shifts in pro- and anti-tumor immune cell populations, emphasizing the critical role of the immune system in these patients. Furthermore, the link between vitamin D levels and CD8 + NK cells in treatment response among overweight patients is a promising area for future research. Although more studies and a larger sample size are needed to explore the influences of excess weight on survival and treatment response, as well as alterations in immune populations.

## Supplementary Information

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Supplementary Material 1.

Supplementary Material 2.

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**Authors' contributions**
L.H.P., D.J.G-D: Conceptualization, data curation, formal analysis, investigation, writing–original draft. C.J-C.: Investigation and conceptualization. E. N-F., N.P.C., A.M.G., E.R.H., J.G.P., M.P.P, A.R.D.: Investigation. L.C-M.: Conceptualization, resources, supervision, funding acquisition, investigation, writing–original draft, project administration. V.S-M.: Conceptualization, supervision, formal analysis, investigation, writing–original draft.

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**Data availability**
Not applicable

## Declarations

**Ethics approval and consent to participate**
The research was conducted according to the International Ethical Guidelines for Biomedical Research Involving Human Subjects, the Declaration of Helsinki, good clinical practice guidelines, and local laws. The study protocol, along with any subsequent amendments, received approval from the Seville Provincial Ethics Committee for Research with Drugs.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare no competing interests.

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