Piemonti Lorenzo (Orcid ID: 0000-0002-2172-2198)
Bosi Emanuele (Orcid ID: 0000-0002-2371-2495)
Pozzilli Paolo (Orcid ID: 0000-0001-5090-636X)
Giorgino Francesco (Orcid ID: 0000-0001-7372-2678)

Ladarixin, an inhibitor of IL-8 receptors CXCR1 and CXCR2, in new-onset type 1 diabetes: a multicenter, randomized, double-blind, placebo-controlled trial

Lorenzo Piemonti[1], Bart Keymeulen[2], Pieter Gillard[3], Thomas Linn[4], Emanuele Bosi[1], Ludger Rose[5], Paolo Pozzilli[6], Francesco Giorgino[7], Efisio Cossu[8], Luisa Daffonchio[9], Giovanni Goisis[9], Pier Adelchi Ruffini[9], Anna Rita Maurizi[9], Flavio Mantelli[9], Marcello Allegretti [9].

[1]IRCCS Ospedale San Raffaele and Università Vita-Salute, Diabetes Research Institute, Milano, Italy;
[2]UZ Brussel Diabetes Clinic, Brussels, Belgium and the Belgian Diabetes Registry;
[3]University Hospitals, Leuven, Belgium;
[4]Universitaetsklinikum Giessen und Marburg GmbH, Giessen, Germany;
[5]Institut fuer Diabetesforschung GmbH, Munster, Germany;
[6]University Campus Bio-Medico, Roma, Italy;
[7]AOU Policlinico Consorziale, Bari, Italy;
[8]AOU Policlinico Monserrato, Cagliari, Italy;
[9]Research and Development, Dompé farmaceutici S.p.A., Milano, Italy

ORCID
LP: 0000-0002-2172-2198; BK: 0000-0002-8671-4527; PG: 0000-0001-9111-4561; TL: 0000-0002-0870-8164; LR: 0000-0003-2424-5857; PP: 0000-0001-5090-636X; FG: 0000-0001-7372-2678; EC: 0000-0002-5269-9285

Short title: Ladarixin in new-onset type 1 diabetes

Correspondence to:

Lorenzo Piemonti, IRCCS Ospedale San Raffaele, Diabetes Research Institute, Via Olgettina 60, 20132 Milan. Fax: 39 02 26432871. Tel: 39 02 26432706. E-mail: piemonti.lorenzo@hsr.it

Word count:
Abstract: 250; Text: 3,317
Table: 1
Figure: 4
Supplementary figure: 1
Supplementary table: 1
References: 27

Declaration of interests. Each study center received research support and compensation from Dompé farmaceutici spa (Milan, Italy) to conduct the study and collect data. LD, GG, PAR, ARM, FM and MA are employees of Dompé farmaceutici spa. LP served as consultant for Dompé farmaceutici spa (Milan, Italy).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/dom.14770

ABSTRACT

Aim. To evaluate the ability of ladarixin, an inhibitor of the CXCR1/2 chemokine receptors (LDX, 400 mg b.i.d for 3 cycles of 14 days on/14 days off) to maintain C-peptide production in adult patients with newly diagnosed type 1 diabetes.

Materials and methods. A double-blind, randomized (2:1), placebo-controlled study was conducted in 45 males and 31 females (18–46 years) within 100 days from the first insulin administration. Primary end-point was the area under the curve for C-peptide in response to a 2-hour MMTT [$AUC_{(0\text{-}120\ min)}$] at week 13±1. Secondary endpoints included C-peptide $AUC_{(15\text{-}120\ min)}$, HbA_{1c}, daily insulin requirement, severe hypoglycaemic events (SHE), the proportion of subjects achieving HbA_{1c} <7.0% without SHE or maintaining a residual beta cell function. Follow-up assessments were scheduled at weeks 13±1, 26±2 and 52±2.

Results. 26/26 (100%, placebo) and 49/50 (98%, LDX) patients completed week 13. Mean change from baseline to week 13 in C-peptide $AUC_{(0\text{-}120\ min)}$ was -0.144±0.449 nmol/L with placebo and 0.003±.322 nmol/L with LDX. The difference was not significant (0.149 nmol/L, 95% CI -0.04 to 0.33; p=0.122). The proportion of patients with HbA1c <7.0% without SHE was transiently (week 26) higher in LDX group (81% vs 54%, p=0.024). Otherwise, no significant secondary endpoint differences were noted. Transient metabolic benefit was seen at week 26 in favour of LDX group in the pre-specified subpopulation with fasting C-peptide < median value at screening.

Conclusions. In newly diagnosed patients with type 1 diabetes, short-term LDX treatment had no appreciable effect on preserving residual beta-cell function.

Trial registration. ClinicalTrials.gov NCT02814838

Funding. This work was funded by Dompé farmaceutici spa (Milan, Italy).

1. INTRODUCTION

Type 1 Diabetes is an immune-mediated chronic disease resulting in a progressive failure of pancreatic beta cells. Despite important improvements in diabetes care in last decades, type 1 diabetes results in short [1] and long-term complications and is one of the leading causes of cardiovascular diseases, end-stage renal disease, blindness and amputations [2]. In spite of more than two decades of efforts and dozens of clinical trial with a variety of immune and non-immune interventions, only five immunotherapies mainly targeting adaptive lymphocyte-mediated attack of beta cells have been shown to preserve insulin secretion in stage 3 type 1 diabetes (teplizumab [3] otelixizumab [4], rituximab [5], abatacept [6], low-dose anti-thymocyte globulin [7], and alefacept [8]) and teplizumab has been shown to delay the onset of stage 2 disease [9]. Type 1 diabetes is generally depicted as a beta cell specific T cell-mediated autoimmune disease, with an associated non beta cell specific inflammatory component [10]. Not surprisingly, some randomized controlled trials targeting innate immune mediators (such as TNFα, IL-1, and IL-6R) have been conducted [11-13]. Recently, neutrophils were proposed as relevant players in the pathogenesis of type 1 diabetes [14]. Pancreas-infiltrating neutrophils were observed at the level of very small blood vessels in the exocrine pancreas of multiorgan donors with type 1 diabetes (both at onset and at later stages of the disease), but not in that of multiorgan nondiabetic donors or donors with type 2 diabetes [15]. A tissue-specific pathogenic role of these pancreas-infiltrating neutrophils is suggested by their ability to extrude neutrophil extracellular traps [16]. Moreover, a mild but significant and reproducible peripheral neutropenia both precedes and parallels the onset of type 1 diabetes [7]. Blood neutrophils in type 1 diabetes revealed a unique molecular signature that is distinguished by an overabundance of IFN-associated genes; despite being healthy, said signature is already present in type 1 diabetes-autoantibody-negative at-risk subjects [16]. The role of neutrophils in the pathogenesis of type 1 diabetes has emerged as pivotal also in the non-obese diabetic (NOD) mice. Diana et al. showed that neutrophils, lymphocytes B-1a, and plasmacytoid dendritic cells are involved in the initiation of the diabetogenic T cell response and autoimmune diabetes development [17]. Moreover, chemokine ligand 8 (CXCL8), commonly called interleukin-8

(IL-8), seems to be an important mediator in the progression of type 1 diabetes, modulating neutrophil trafficking and recruitment through specific CXCR1 and CXCR2 receptors [18]. Indeed, we showed that the inhibition of the neutrophil recruitment by ladarixin (LDX), an allosteric inhibitor of IL-8 receptors CXCR1/CXCR2 [19], could prevent and revert the hyperglycemia in the NOD mouse. This evidence provided the basis for this phase 2 safety and efficacy study of LDX in newly diagnosed type 1 diabetes patients, testing the ability of the drug to preserve beta cell function and delay further disease progression.

2. MATERIALS AND METHODS

2.1 Study design and patients. This phase 2 clinical trial was registered with ClinicalTrials.gov (NCT02814838) and conducted in compliance with all applicable regulatory requirements. This was a multicentre, randomized, double-blind, parallel-assignment study conducted at eight EU centres (four in Italy, two in Germany and two in Belgium) in newly diagnosed type 1 diabetes patients. Since there were no data available to estimate the effect size of LDX in type 1 diabetes patients, the sample size for this study was based on figures provided by Lachin [20], considering an adult population (> 18 years) and the log(x+1) transformed C-peptide AUC from the Mixed Meal Tolerance Test (MMTT), initially selected by TrialNet as the appropriate transformation. With these assumptions, 72 patients were planned to be included in the trial, to provide 85% power to detect a 50% between-group difference (α=0.05, 1-sided) in the 2-hour MMTT C-peptide AUC [$AUC_{(0\text{-}120\ min)}$], assuming a 24% drop-out rate. As a minimum, inclusion criteria included: age 18-45 years, new-onset (randomization within 100 days from the first insulin administration) type 1 diabetes confirmed by at least one positive diabetes-related autoantibody (anti-GAD [GADA], anti-insulin [IAA], anti-IA-2 [IA-2A] or anti-ZnT8 [ZnT8A]), insulin requirement at some time and residual beta cell function as per peak stimulated (MMTT) C-peptide level >0.2nmol/L. Exclusion criteria included: patient taking pre-mixed insulin or on insulin pump, creatinine clearance <60 mL/min, ALT/AST >3 x ULN and total bilirubin >3 mg/dL, hypoalbuminemia (serum albumin <3 g/dL), QTcF >470 msec, and other

significant comorbid conditions or administration of concomitant medications that could have biased the efficacy outcome/readout.

2.2 Study treatment, randomization and masking. Patients received hard gelatine capsules of either LDX at the dose of 400 mg twice a day for 3 cycles of 14 days on/14 days off, or placebo (same schedule), according to his/her randomization number (Supplementary Figure 1). LDX inhibits neutrophil (PMN) migration in vitro with an IC50 in the range of 1 ng/mL, as per pre-clinical data. PK trials in humans has established that the 400 mg dose provide an average steady state plasma concentration of the ladarixin unbound fraction of about 100-150 ng/mL. As a consequence, the 400 mg dose has been selected to ensure full inhibition of PMN migration. The two daily doses were administered orally in the morning and in the evening, 2 hours apart from breakfast and dinner, respectively. An independent statistician generated the master randomization list, balancing LDX and placebo in a 2:1 fashion within each centre. Individual treatment codes were provided as sealed envelopes to the investigators and sponsor pharmacovigilance for emergency/safety purposes. To maintain blindness, the appearance of the capsules, including packaging and labelling, did not allow the recognition of the actual treatment (either LDX or placebo).

2.3 Procedures and endpoints. Patients enrolled in this trial were admitted to an intensive diabetes management, according to ADA recommendation, to ensure optimal glycaemic control. Insulin therapy was based on multiple daily insulin injections. Patients were instructed to self-monitor (finger-stick) their glucose values at least 4 times per day to allow insulin to be titrated up or down to the following targets: pre-prandial blood glucose of 70-130 mg/dL, post-prandial blood glucose < 180 mg/dL, and bed-time blood glucose of 110-150 mg/dL, consistent to an overall target of HbA_{1c} <7%. Screening included evaluation of medical history and disease-specific clinical information, including the date of first insulin administration and autoantibody status (at least one positive among GADA; IAA, if obtained within 10 days of insulin therapy; IA-2A and ZnT8A) to confirm type 1 diabetes diagnosis. Baseline daily insulin requirement, HbA_{1c}, C-peptide and glucose from the

MMTT were assessed within 3 weeks before randomization. Follow-up assessments were scheduled at weeks 13±1 (month 3), 26±2 (month 6) and 52±2 (month 12) from the beginning of treatment. Pre-specified primary outcome was the Area Under the Curve (AUC) for the serum C-peptide level during 2 hours [$AUC_{(0\text{-}120\ min)}$] of an MMTT at weeks 13±1. Secondary endpoints included MMTT C-peptide increase above fasting values [$AUC_{(15\text{-}120\ min)}$], HbA_{1c}, daily insulin requirement, severe hypoglycaemic events (SHE), the proportion of subjects achieving an HbA_{1c} <7.0% without SHE and the proportion of patients maintaining a residual beta cell function (defined as at least one MMTT C-peptide value ≥0.2 nmol/L). Incidence of treatment emergent adverse event, vital signs and standard laboratory parameters (haematology and clinical chemistry) were specific safety endpoints.

2.4 Statistical analysis. Data are presented as mean ± standard deviation (SD) or median, according to their distribution. All the AUC analyses were based on actual rather than scheduled timings and were calculated using the trapezoidal rule. Analyses were performed according to the intention-to-treat (ITT) principle; all statistical tests were performed 1-sided with α=0.05, unless otherwise specified. The $AUC_{(0\text{-}120\ min)}$ after the MMTT at Week 13±1 was transformed as log(x+1) values; transformed AUC was analyzed with an ANCOVA model adjusting for sex, baseline age, and baseline C-peptide $AUC_{(0\text{-}120\ min)}$ and unpaired T test. The comparisons between treatment groups on log(x+1) transformed $AUC_{(0\text{-}120\ min)}$, percent change from baseline of $AUC_{(0\text{-}120\ min)}$, average daily insulin requirement, and HbA_{1c} value were carried-out using a mixed linear model with treatment group, visit, treatment by visit interaction as fixed factors of the model and patient as random effect. Number and proportion along the 95% CI (Clopper-Pearson's formula) of patients with HbA_{1c} <7% and absence of SHE from the previous visit were calculated for each time point. The comparison between the two study treatment groups was performed by means of a Fisher's exact text at each time point. Alternative approaches, including subset analysis and AUC geometric mean ratios, were explored, as described in the sections below.

2.5 Study approval. The protocol, protocol amendments, and consent documents were approved by appropriate Ethics Committees. All participants provided written, informed consent.

3. RESULTS

3.1 Patient disposition and baseline characteristics. The predefined ITT cohort included all the 76 patients who underwent randomization and received at least one dose of study medication. Details of patient disposition and inclusion in analysis sets are shown in Figure 1. One patient out of 76 randomized did not complete the 13 weeks MMTT (because of early withdrawal of consent); thus, 75 patients were included in the primary outcome analysis (49 on LDX, 26 on placebo). Seventy-three out of 75 patients completed week 52 follow-up (48 on LDX, 25 on Placebo): one patient on placebo discontinued from the study due to consent withdrawal; one patient on LDX was lost to follow-up. Demographic characteristics of the ITT patients are reported in Table 1. Mean exposure (percent of scheduled total dose) to LDX was 97.7±7.6%. This includes two patients with a study treatment compliance <80%. The majority of patients were positive for two or more autoantibodies, being GADA the most frequent, followed by ZnT8A. There were no notable differences between treatment groups with respect to demographic and baseline characteristics. Neutrophil count was comparable at screening in the two treatment groups (LDX 3.37±1.21 10^9/L, placebo 3.25 ± 1.16 10^9/L) and remained as such at week 13 (treatment completion: LDX 3.49 ± 1.56 10^9/L, placebo 3.32 ± 1.23 10^9/L).

3.2 Efficacy outcomes. MMTT stimulated C-peptide $AUC_{(0\text{-}120\ min)}$ adjusted for age, sex, and baseline C-peptide value was similar between the groups at 13 weeks (LDX 4.03 nmol/L, 95% CI 3.89 to 4.16; and placebo 3.87 nmol/L, 95% CI 3.54 to 4.15, Figure 2). The difference was not significant (mean 0.14 nmol/L, 95% CI -0.14 to 0.42; p=0.122 ANCOVA; t-test, 2 sided p=0.33). Specifically, the results of the linear mixed model for the $AUC_{(0\text{-}120\ min)}$ over the study showed statistically significant effects over time (p<0.0001), while the factor treatment (p=0.6928) and the interaction treatment by visit (p=0.0993) were not statistically significant. Result on primary outcome were not impacted by including in the analysis the time from first insulin to treatment (p=0.2035, ANCOVA), even if the time itself was statistically significant at 0.05 level (p=0.0497, ANCOVA)

Similarly the adjusted mean difference between LDX and placebo for the C-peptide $AUC_{(15\text{-}120\ min)}$ was not statistically significant over the study. The mean (±SD) insulin requirement at screening was 0.325 (±0.1923) IU/kg/day for the LDX group and decreased at week 13 [-0.067 (±0.1774) IU/kg/day]; however, an increasing trend was seen at week 26 [-0.011 (±0.2625) IU/kg/day] and week 52 [0.025 (±0.2507) IU/kg/day]. A similar profile was seen in the placebo group. The linear mixed model of daily insulin requirement over the study showed statistically significant effect over time ($p<0.0001$), while the factor treatment ($p=0.3668$) and the interaction treatment by visit ($p=0.7121$) were not statistically significant. The adjusted HbA_{1c} mean differences between LDX and placebo were not statistically significant over the study. A maximum decrease in HbA_{1c} level was seen at week 13 compared with week 26 and week 52 in both the treatment groups. The results of the linear mixed model on HbA_{1c} showed statistically significant effect over time ($p<0.0001$), while the factor treatment ($p=0.8988$) and the interaction treatment by visit ($p=0.4588$) were not statistically significant. The proportion of patients with HbA_{1c} <7% in the absence of SHE is reported as a composite endpoint in figure 2. The overall mean cumulative SHE/patient occurring from randomisation was 0.1 in the LDX group (2 patients) and 0.1 in the placebo group (one patient). The comparison between treatment groups for the proportion of patients with HbA_{1c} <7% and absence of SHE was statistically significant at week 26 ($p=0.0248$) in favour of the LDX group [LDX=39 patients (81.3%) vs. placebo=13 patients (50%)] and a trend was also evident at week 13 ($p=0.0779$). The results at week 26 was confirmed also by a logistic regression model which included time elapsed from first insulin injection ($p=0.0087$). The proportion of patients maintaining a residual beta cell function over the study is also presented in Figure 2. At week 52, 78% of patients maintained a residual beta cell function in the LDX group, as compared to 76.9% in the placebo group. The comparison between treatment groups was not statistically significant over the study.

Predefined subgroup analyses were performed on the efficacy endpoints according to age class (<25 and ≥25 years), fasting C-peptide (pre-MMTT) (<median value and ≥median value) and the number (from 1 to 4) of positive autoantibodies at screening/diagnosis (Figure 3). In patients with fasting C-peptide at screening <0.205 nmol/L (median value), the difference in the C-peptide $AUC_{(15\text{-}120\ min)}$ between LDX (n=26) and placebo (n=11) reached statistical significance at week 26 (LDX=3.22±0.55 vs placebo=2.53±1.18, adjusted mean differences 0.6304 CI 0.061-1.199; p=0.031) (Figure 4). Accordingly, the proportion of patients with HbA_{1c} <7% in the absence of SHE was significantly higher at week 26 for patient receiving LDX as compared to placebo (LDX=88.5% vs placebo=36.4%; p=0.0074). Moreover, clear trends were evident in favor of LDX for HbA_{1c} at week 26 [LDX=6.3% (CI 6.1-6.5) vs placebo=7.01% (CI 5.8-8.1), p=0.053] and for the proportion of patients maintaining a residual beta cell function at week 13 (LDX=100.0% vs placebo=81.8%, p=0.0826) and 26 (LDX=95.8% vs placebo=70.0%, p= 0.0666) (Figure 4). No statistically significant changes were observed between the two treatments in the other subgroups, with the exception of the proportion of patients maintaining a residual beta cell function at week 13 in patients aged ≥25 years that was higher in the LDX group than in placebo (LDX=100% vs placebo=81.3%, p=0.0345).

3.3 Adverse events and safety. Overall, a good safety profile was observed for LDX. It was safe and well tolerated and no clinically relevant safety observations were detected (Supplementary table 1). Specifically, no differences between treatment groups were observed for rates, severity and distribution of TEAEs or TESAEs. Thirty-seven patients (74.0%) in the LDX group and 21 patients (80.77%) in the placebo group reported at least one TEAE during study participation. The majority of the TEAEs reported in the study were considered mild in severity. The most common TEAEs presented by primary SOC were infections and infestations (LDX=46.0% vs. placebo=46.2%), followed by gastrointestinal disorders (LDX=36.0% vs. placebo=34.6%) and nervous system disorders (LDX=34.0% vs. placebo=26.9%). TEAEs were considered related to study treatment (Adverse Drug Reactions – ADRs) in 20 patients (40.0%) in the LDX group (52 ADRs) and 8 patients

(30.8%) in the placebo group (17 ADRs). ADRs occurring in ≥10% of patients included dyspepsia (LDX=16% vs placebo=0%) and headache (LDX=16% vs placebo=15.4%). A total of 3 patients in the LDX group and one patient in the placebo group reported TESAEs, none of which was considered related to the study treatment. TEAEs led to treatment discontinuation in one patient (2%) in the LDX group because of ALT/AST increase, and in one patient (3.8%) in the placebo group because of rash (Supplementary table 1). No patient died in the study. There were no clinically meaningful changes in mean values from screening to assessment time points for hematology and blood chemistry parameters across the treatment groups. Analyses of vital signs did not reveal any clinically relevant differences across the treatment groups.

4. DISCUSSION

Our results showed that a short-term transient inhibition of IL-8 receptors CXCR1/CXCR2 with the allosteric inhibitor LDX [21] did not consistently slow the decline in beta cell function in recent-onset type 1 diabetes adults. The use of LDX in type 1 diabetes was proposed on the basis of preclinical evidences where transient blockade of CXCR1/CXCR2 was effective in preventing the inflammatory damage in the mouse model of multiple low-dose streptozotocin injections and in preventing and reversing diabetes in NOD mice [18]. In addition to the well-known limitations of the preclinical models in predicting the success of treatments at the onset of type 1 diabetes in humans, more than one hypothesis can explain the outcome difference between preclinical studies and this human trial. In mouse models, LDX consistently decreased the percentage of pancreas-infiltrating polymorphonuclear cells and modulated the distribution of various leukocyte populations targeting CXCR2+ leukocyte subpopulations, which are known to be required for the initiation of beta cell disruptive insulitis in both mouse and human type 1 diabetes [10,15-18,22]. In support of this, the most extensive anti-inflammatory changes and the highest efficacy of LDX were observed in the 12-week-old NOD mice, a disease phase characterized by marked beta cell disruptive insulitis immediately preceding the onset of hyperglycemia. In this trial, LDX treatment was limited to 3 cycles of 14 day on/14 days off, in compliance with regulatory requirements based on the preclinical safety data available at the time of trial submission. Consistently, considering that polymorphonuclear cells are short half-life cells, we chose the end of treatment (13 weeks) as the time to evaluate the primary end point. This schedule was probably undersized to keep beta cell specific autoimmunity in humans under control. In support of this hypothesis, also in the preclinical model a 14 day treatment rapidly reverted diabetes in 78% of animals but did not assure a long-term benefit on glucose levels and the disease as quicker recurred as higher the glycaemia was at onset. More generally, the inability to identify the time in which the local inflammatory response is at its peak may have contributed to the negative result. The absence of validated biomarkers of islet inflammation may represent a problem for any clinical study targeting pancreatic inflammation in type 1 diabetes, limiting the ability to

anticipate trial enrollment and treatment initiation versus the pre-disease onset phase. In line with this observation, other anti-inflammatory strategies have been tested in type 1 diabetes patients at the onset of the disease. Findings from small pilot clinical trials suggested that inhibition of IL-1 [23], TNF-α [24] or IL-6 signals might have a beneficial effect in type 1 diabetes, but the results were only partially confirmed in randomized phase 2 trials [12,25 26]. On the other hand, five immunotherapies mainly targeting adaptive lymphocyte-mediated attack of beta cells have been shown to preserve insulin secretion in stage 3 type 1 diabetes [teplizumab [3] otelixizumab [4], rituximab [5], abatacept [6], low-dose anti-thymocyte globulin [7], and alefacept [8]] and teplizumab has been shown to delay the onset of stage 2 disease [9]. However, the transient nature of the efficacy observed or the associated side effects, or both, have until now prevented the marketing approval of these therapies. In our study, LDX showed an excellent tolerability profile and no safety issues emerged. While the complexity of type 1 diabetes and of its clinical management is clear, further research should not be discouraged in order to attempt to halt disease progression, possibly also considering combination therapies with agents presenting a favorable safety profile. In this context, an orally bioavailable small molecule such as the CXCR1/CXCR2 inhibitor LDX constitutes an opportunity for testing a second generation of disease-modifying treatment in type 1 diabetes [27]. The study is under-powered to detect a true difference in the subpopulation analysis. Despite this, some transient metabolic benefits were seen in favour of LDX group, in particular in patients with lower fasting C-peptide and higher insulin requirement at screening. Further metabolic and immunologic studies are ongoing and will allow to better understand the meaning of such findings, but the more pronounced positive effect observed in a population with lower fasting C-peptide at screening, that is also characterized by a severely impaired metabolic function, may suggest the existence of a clinical condition (either a population or a disease stage) responsive to IL-8 inhibition. Nevertheless, together with the duration of treatment, the study has some other limitations that are consistent with phase 2 trials, like the small number of participants and the limited range of age within the studied population. Moreover, children were not included in the study, due to the early development phase, so the impact in younger ages that may have different

progression characteristics is not known. In conclusion, short-term transient inhibition of CXCL8 receptors CXCR1/CXCR2 with the allosteric inhibitor LDX did not consistently slow the decline in beta cell function in newly diagnosed patients with type 1 diabetes. The lack of response to LDX in this study suggests that the role of IL- 8 and its receptors CXCR1/2 in T1D is complex. Therapeutic interventions targeting IL- 8 and its receptors CXCR1/2 in the future may be most beneficial extending the exposure to LDX or its use in combination with therapies that synergize with the IL-8–driven pathways most important in T1D pathogenesis.

Author contributions

LP: Conceptualization, Methodology, Formal analysis, Writing - Original Draft. BK: Investigation, Methodology, Review & Editing. PG: Investigation, Review & Editing. TL: Investigation, Review & Editing. EB: Investigation, Review & Editing. LR: Investigation, Review & Editing. PP: Investigation, Review & Editing. FG: Investigation, Review & Editing. EC: Investigation, Review & Editing. LD: Conceptualization, Methodology. GG: Formal analysis. PAR: Writing - Review & Editing. ARM Writing - Review & Editing. FM: Resources. MA Resources. LP is the guarantor of this work and, as such, had full access to all the data presented in the study and takes responsibility for their integrity and for the accuracy of data analysis. The final manuscript has been read and approved by all named authors.

Acknowledgments

This work was funded by Dompé farmaceutici spa (Milan, Italy). Each study center received research support and compensation from Dompé farmaceutici spa (Milan, Italy) to conduct the study and collect data. LP is the guarantor of this work and, as such, had full access to all the data presented in the study and takes responsibility for their integrity and for the accuracy of data analysis. The final manuscript has been read and approved by all named authors. NO data outside those published in this manuscript and the material presented in the supplemental material are available for sharing.

REFERENCES

1. Sauder KA, Stafford JM, Mayer-Davis EJ, et al. Co-occurrence of early diabetes-related complications in adolescents and young adults with type 1 diabetes: an observational cohort study. *The Lancet Child & Adolescent Health*. 2019;3(1):35-43.
2. Perkins BA, Lovblom LE, Lanctot SO, Lamb K, Cherney DZI. Discoveries from the study of longstanding type 1 diabetes. *Diabetologia*. 2021;64(6):1189-1200.
3. Herold KC, Gitelman SE, Willi SM, et al. Teplizumab treatment may improve C-peptide responses in participants with type 1 diabetes after the new-onset period: a randomised controlled trial. *Diabetologia*. 2013;56(2):391-400.
4. Keymeulen B, Vandemeulebroucke E, Ziegler AG, et al. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med*. 2005;352(25):2598-2608.
5. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N Engl J Med*. 2009;361(22):2143-2152.
6. Orban T, Bundy B, Becker DJ, et al. Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2011;378(9789):412-419.
7. Haller MJ, Long SA, Blanchfield JL, et al. Low-Dose Anti-Thymocyte Globulin Preserves C-Peptide, Reduces HbA1c, and Increases Regulatory to Conventional T-Cell Ratios in New-Onset Type 1 Diabetes: Two-Year Clinical Trial Data. *Diabetes*. 2019;68(6):1267-1276.
8. Rigby MR, DiMeglio LA, Rendell MS, et al. Targeting of memory T cells with alefacept in new-onset type 1 diabetes (T1DAL study): 12 month results of a randomised, double-blind, placebo-controlled phase 2 trial. *Lancet Diabetes Endocrinol*. 2013;1(4):284-294.
9. Herold KC, Bundy BN, Long SA, et al. An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes. *New England Journal of Medicine*. 2019;381(7):603-613.
10. Citro A, Dugnani E, Campo F, Piemonti L. Innate immunity mediated inflammation and beta cell function: neighbors or enemies? *Frontiers in Endocrinology*. 2020;11:1129.

11. Mastrandrea L, Yu J, Behrens T, et al. Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. *Diabetes Care.* 2009;32(7):1244-1249.

12. Moran A, Bundy B, Becker DJ, et al. Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. *The Lancet.* 2013;381(9881):1905-1915.

13. Hundhausen C, Roth A, Whalen E, et al. Enhanced T cell responses to IL-6 in type 1 diabetes are associated with early clinical disease and increased IL-6 receptor expression. *Science translational medicine.* 2016;8(356):356ra119-356ra119.

14. Huang J, Xiao Y, Xu A, Zhou Z. Neutrophils in type 1 diabetes. *Journal of diabetes investigation.* 2016;7(5):652-663.

15. Valle A, Giamporcaro GM, Scavini M, et al. Reduction of circulating neutrophils precedes and accompanies type 1 diabetes. *Diabetes.* 2013;62(6):2072-2077.

16. Vecchio F, Buono NL, Stabilini A, et al. Abnormal neutrophil signature in the blood and pancreas of presymptomatic and symptomatic type 1 diabetes. *JCI Insight.* 2018;3(18).

17. Diana J, Simoni Y, Furio L, et al. Crosstalk between neutrophils, B-1a cells and plasmacytoid dendritic cells initiates autoimmune diabetes. *Nature Medicine.* 2013;19(1):65-73.

18. Citro A, Valle A, Cantarelli E, et al. CXCR1/2 inhibition blocks and reverses type 1 diabetes in mice. *Diabetes.* 2015;64(4):1329-1340.

19. Bertini R, Barcelos L, Beccari A, et al. Receptor binding mode and pharmacological characterization of a potent and selective dual CXCR1/CXCR2 non-competitive allosteric inhibitor. *British journal of pharmacology.* 2012;165(2):436-454.

20. Lachin JM, McGee PL, Greenbaum CJ, et al. Sample size requirements for studies of treatment effects on beta-cell function in newly diagnosed type 1 diabetes. *PLoS One.* 2011;6(11):e26471.

21. Allegretti M, Cesta MC, Garin A, Proudfoot AE. Current status of chemokine receptor inhibitors in development. *Immunology letters*. 2012;145(1-2):68-78.

22. Niu S, Bian Z, Tremblay A, et al. Broad Infiltration of Macrophages Leads to a Proinflammatory State in Streptozotocin-Induced Hyperglycemic Mice. *J Immunol*. 2016;197(8):3293-3301.

23. Sumpter KM, Adhikari S, Grishman EK, White PC. Preliminary studies related to anti-interleukin-1β therapy in children with newly diagnosed type 1 diabetes. *Pediatric diabetes*. 2011;12(7):656-667.

24. Peters MJ. Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study: response to Mastrandrea et al. *Diabetes Care*. 2009;32(12):e153; author reply e154.

25. Quattrin T, Haller MJ, Steck AK, et al. Golimumab and Beta-Cell Function in Youth with New-Onset Type 1 Diabetes. *New England Journal of Medicine*. 2020;383(21):2007-2017.

26. Greenbaum CJ, Serti E, Lambert K, et al. IL-6 receptor blockade does not slow β cell loss in new-onset type 1 diabetes. *JCI Insight*. 2021;6(21):e150074.

27. von Herrath M, Bain SC, Bode B, et al. Anti-interleukin-21 antibody and liraglutide for the preservation of β-cell function in adults with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled, phase 2 trial. *The lancet Diabetes & endocrinology*. 2021;9(4):212-224.

Figure legends

Figure 1. Enrollment, Randomization, and Follow-up of Study Participants. Between August 2016 and May 2018, 85 new-onset type 1 diabetes patients were assessed for eligibility and 76 were randomized. All randomized patients were included in the intention-to-treat cohort

Figure 2. Trial primary and secondary outcomes. Effects of LDX on 2-hour Area Under the Curve (AUC) of C-peptide ($AUC_{(0\text{-}120\ min)}$), C-peptide $AUC_{(15\text{-}120\ min)}$ above fasting value, HbA_{1c} level, insulin dose, proportion of patients with HbA_{1c} <7% and absence of episodes of severe hypoglycemia (SHE) and proportion of patients maintaining a residual beta cell function (defined as at least one MMTT C-peptide value ≥ 0.2 nmol/L). Means (95% CI) or proportions for each treatment group are reported over time. The analysis of covariance model adjusted for age, sex, baseline value, and treatment assignment or Fisher's Exact Test for categorical independent variables were used to compare the two groups. All p values referring to week 13 are reported in full. * $p<0.05$

Figure 3 a-c. Subgroup plot of ratios for effect of treatment on mean AUC C-Peptide at 13±1 (month 3), 26±2 (month 6) and 52±2 (month 12) from the beginning of treatment. Ratio of geometric means for LDX versus placebo , with 95% confidence intervals, within subgroups of patients as defined at the baseline. When adjusted for multiple subgroup analyses, there was no significant heterogeneity (test of treatment by subgroup interaction) among subgroups. When considering the subgroup with fasting C-peptide <median value (0.205 nmol/L), the 71% improvement seen with LDX versus control at 26 weeks was nominally significant (p=0.033, not adjusted for multiple tests) while not significant in the other subgroups or at week 13 and 52.

Figure 4. Primary and secondary outcomes in predefined subgroup with fasting C-peptide (pre-MMTT) <0.205 nmol/L (median value). Effects of LDX on 2-hour Area Under the Curve (AUC) of C-peptide ($AUC_{(0\text{-}120\ min)}$), C-peptide $AUC_{(15\text{-}120\ min)}$ above fasting value, HbA_{1c} level, insulin dose, proportion of patients with HbA_{1c} <7% and absence of episodes of severe hypoglycemia (SHE) and proportion of patients maintaining a residual beta cell function (defined as at least one MMTT C-peptide value > 0.2 nmol/L). Means (95% CI) or proportions for each treatment group are reported

over time. The analysis of covariance model adjusted for age, sex, baseline value, and treatment assignment or Fisher's Exact Test for categorical independent variables were used to compare the two groups. All p values referring to week 13 are reported in full. * $p<0.05$, ** $p<0.001$

TABLE

Table 1. Characteristics of the study groups

	LDX (N=50)	Placebo (N=26)
Age (years) Mean Median Range	 27.6± 7.06 26 18-46	 26.8±6.35 26.5 18-38
Male sex [N (%)]	29 (58)	16 (61.5)
Ethnic group [N (%)] White/Caucasian	 49 (98)	 26 (100)
No. of autoantibodies [N (%)] 1 2 3 4	 7 (14) 19 (36) 13 (28) 11 (22)	 4 (15.4) 7 (26.9) 7 (30.8) 7 (26.9)
IAA+ GADA IA-2A ZnT8	21 (42) 47 (94) 28 (56) 32 (64)	11 (42.3) 23 (88.5) 17 (65.4) 19 (73.1)
No. of days from first insulin to treatment Median Range§	 74 29-104	 77 40-107
Weight (kg)	68.52 (47.2-110.4)	68.27 (44-109.2)
BMI	22.5 (18.2-34.5)	22.7 (18.8-30.8)
White Blood Cells (cells/mm3)	5.95 ± 1.54	5.77 ± 1.29
Creatinine (μmol/L)	71.8 ± 13.58	66.4 ± 10.91
Creatinine clearance (mL/min)*	127.3 ± 31.55	137.1 ± 35.34
Fasting C-peptide (nmol/L)	0.218 ± 0.1087	0.225 ± 0.1416
Peak stimulates C-peptide (nmol/L)	0.676 ±0.2708	0.675 ± 0.2882
C-peptide $AUC_{(0-120)}$ (nmol/L)	60.381 ± 24.9210	59.092 ± 26.243
HbA_{1c} (mmol/mol, (%))	60 (7.60 ± 1.62)	50 (7.50 ± 1.37)
$HbA_{1c} \geq 7\%$ [N (%)]	28 (56)	15 (57.7)
Insulin requirement (U/kg/day)	0.33 ± 0.192	0.33 ± 0.198

All are means±SD, unless otherwise specified.

§: one patient in each treatment group was randomized slightly after 100 days from the first insulin injection (day 103 and day 106 in the LDX e placebo group, respectively); exemption was granted due to patients being already committed to study participation. Such a delay was not considered to impact trial outcome.

*: Cockcroft-Gault formula

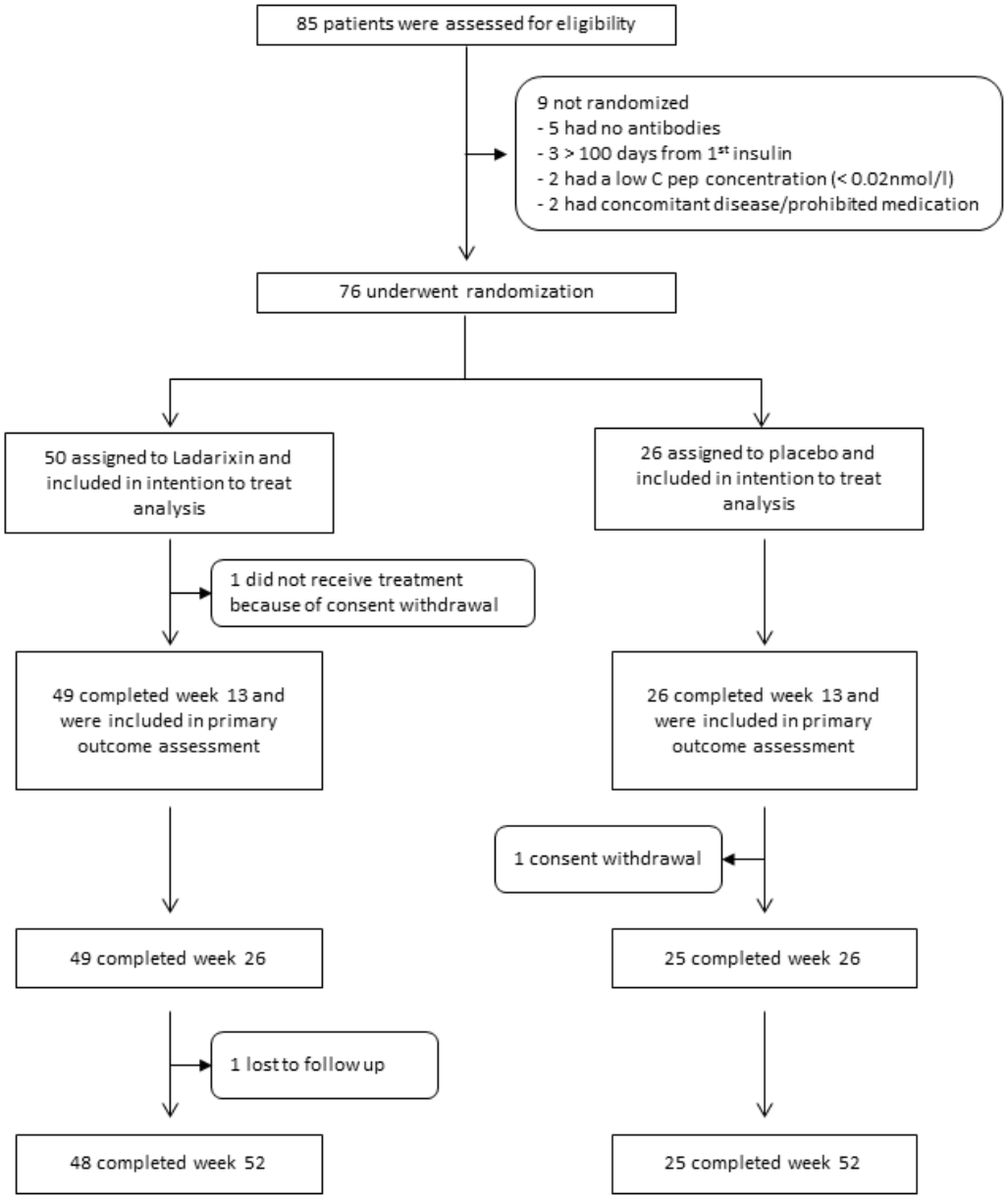


Figure 1

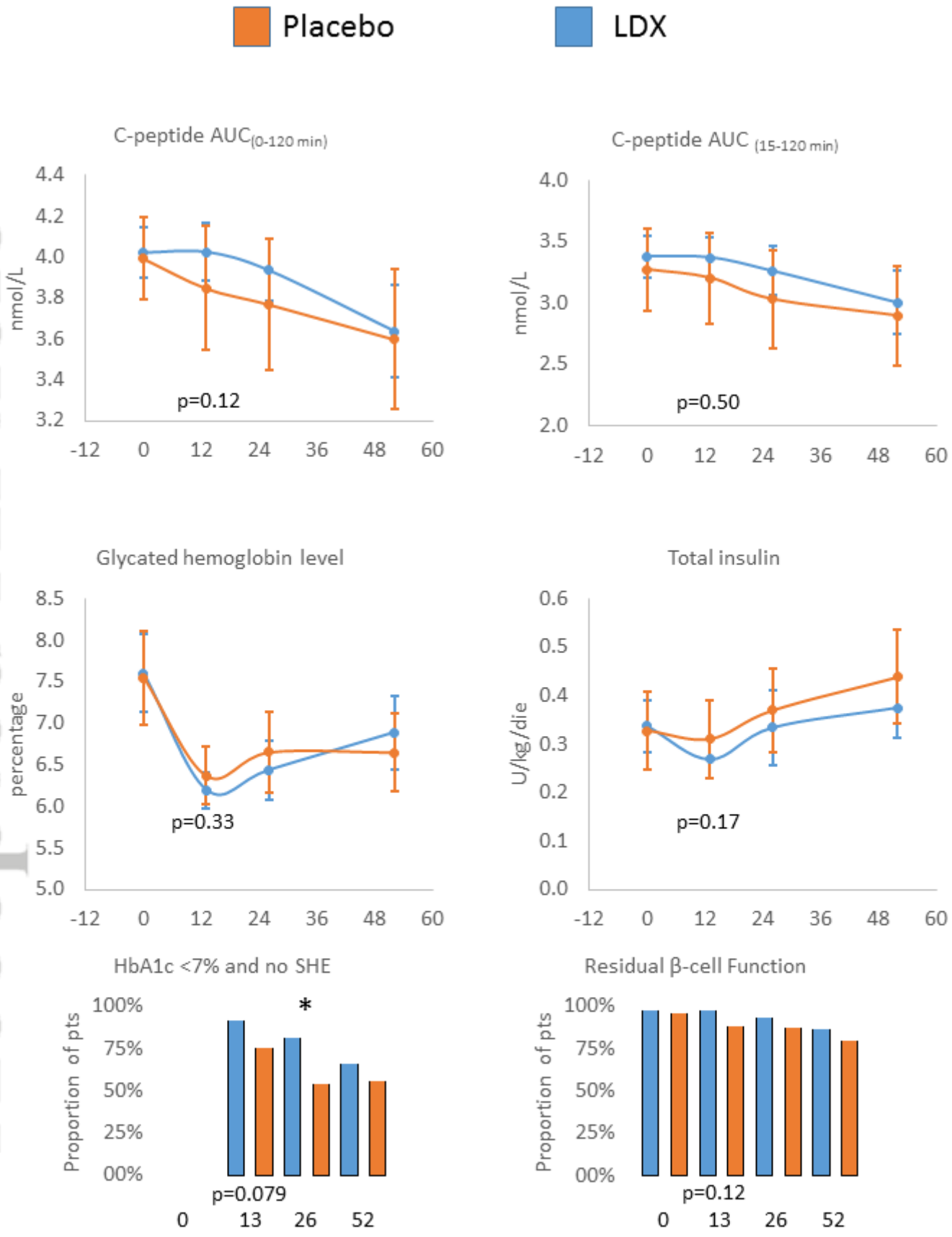
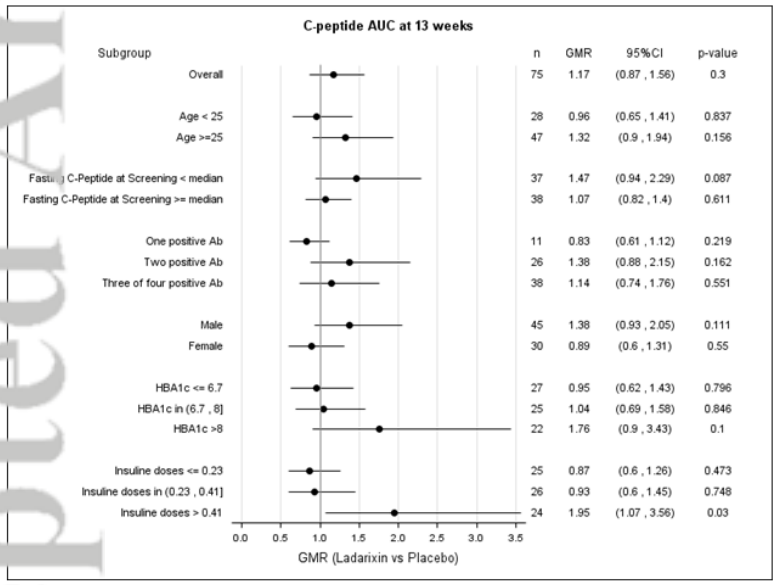
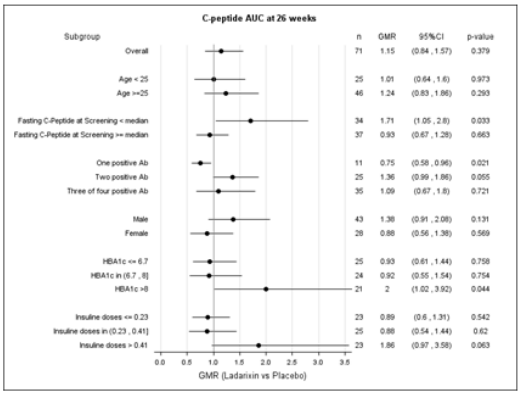


Figure 2

A



B



C

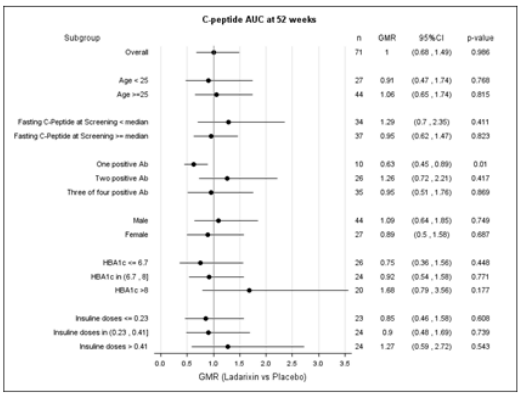


Figure 3

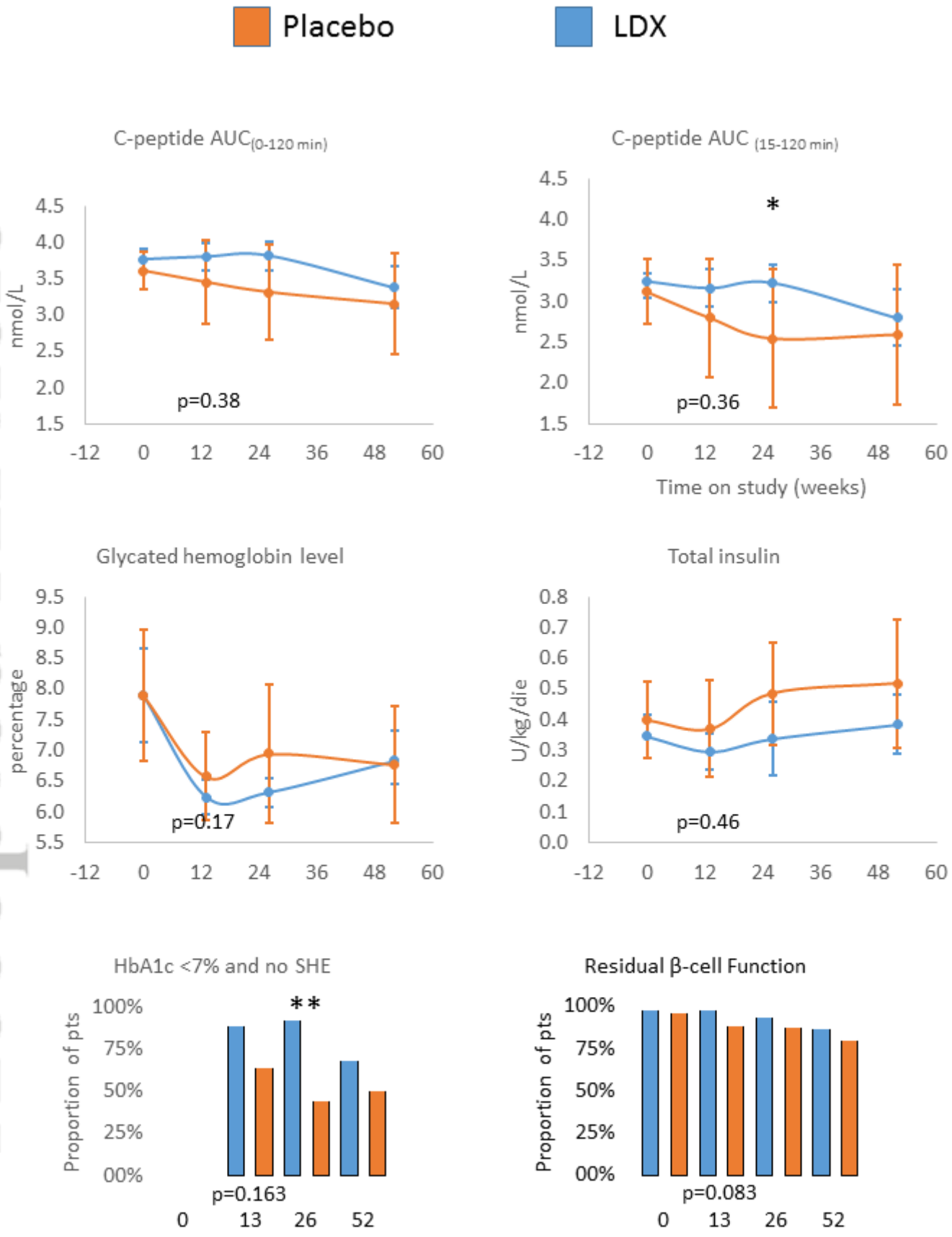


Figure 4