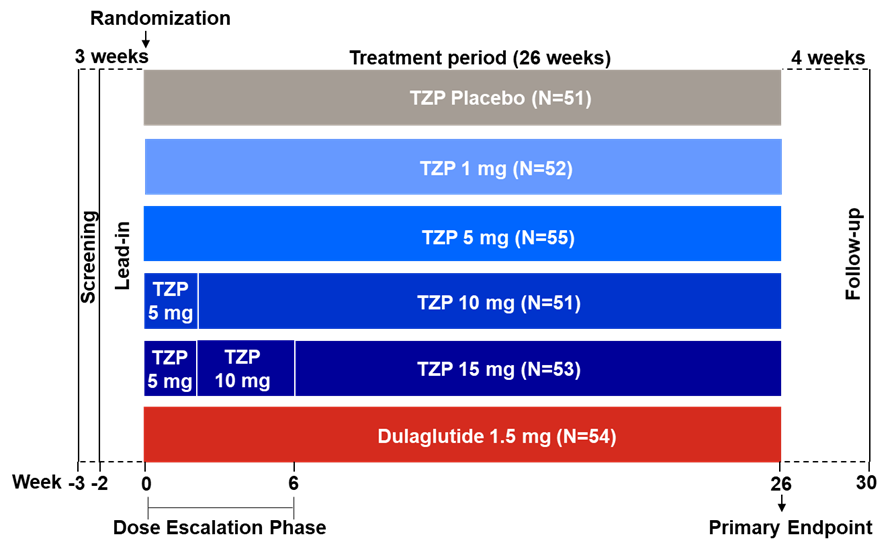
**ONLINE-ONLY SUPPLEMENTAL MATERIAL**

**Supplemental Appendix.**

**Study Design and Participants**

Eligible participants (aged 18–75) had type 2 diabetes for at least 6 months (HbA1c 7.0–10.5%, inclusive), that was inadequately controlled with diet and exercise alone or with stable metformin therapy, and a BMI of 23–50 kg/m². The full list of inclusion and exclusion criteria is published in the Supplementary Appendix of Frias et al. 2018 (1). During the lead-in period, eligible patients were to continue their pre-study therapy, metformin (same formulation and dose), in order to allow reliable assessment of HbA1c at baseline. Eligible patients were randomized in a 1:1:1:1:1:1 ratio to one of the treatment groups: placebo, tirzepatide 1 mg, tirzepatide 5 mg, tirzepatide 10 mg, tirzepatide 15 mg, or dulaglutide 1.5 mg. The 26-week treatment period consisted of a 2- to 6-week dose escalation phase for the tirzepatide 10 mg and 15 mg groups, followed by a 20- to 26-week dose maintenance treatment phase at the final dose. Supplemental Figure 1 illustrates the study design. The primary objective of the Phase 2b study was to demonstrate a dose-response relationship of tirzepatide on HbA1c change from baseline relative to placebo in patients with T2DM. The key secondary objective was to determine the effect of tirzepatide on mean body weight change from baseline to 12 and 26 weeks compared with dulaglutide and placebo.

**Supplemental Figure 1. Study Design**



**Biomarkers**

Fasting serum ALT, AST, and total adiponectin were measured as clinical protocol study variables (Pacific Biomarkers Inc., Seattle, WA, USA) at baseline and after 12 and/or 26 weeks of treatment. Adiponectin is an adipocyte-specific protein with potential roles in glucose and lipid homeostasis; it has been reported to have antifibrogenic and antisteatogenic effects in the liver. Although normal ranges for total adiponectin are not well-defined, concentrations are known to be lower in patients with obesity, T2DM and NAFLD (2-4). Post-hoc exploratory EDTA plasma biomarkers K-18 M30 fragment (VLVbio, Nacka, Sweden) and Pro-C3 (Nordic Bioscience A/S, Herlev, Denmark) were also measured at the same fasting timepoints. The caspase cleavage M30 fragment (neo-epitope at the aspartic acid residue 396) of the intermediate filament K-18 is an early event during apoptosis of hepatocytes (5). Median normal healthy donor control values of 187 U/L were measured with an average intra-assay percent coefficient of variation of 11.5% across the study. Pro-C3 epitope is the released N-terminal pro-peptide of type III collagen resulting from cleavage between residues 145 and 153 (PTGPQNYSP) and is a marker of collagen III formation (6). Circulating levels in post-menopausal women donor levels range from 5.3 to 17.6 ng/mL with an average intra-assay percent coefficient of variation of <8.2%.

**Additional Information on Statistical Methods for Data Presented in Supplemental Tables S3-S7**

To explain the contribution of relevant confounding variables to the variability of the change from baseline in NASH-related biomarkers, stepwise variable selection based on Akaike Information Criterion (AIC) (7, 8) was performed on the pooled data from all tirzepatide doses. The confounding variables included in the models were: weight (baseline and change), HbA1c (baseline and change), triglycerides (change), fasting insulin (change), age, sex and baseline value of the biomarker. To decide if the biomarker needed to be log transformed due to skewness, criteria comprised of the Shapiro-Wilk test and Kurtosis evaluation, before and after taking the log, were applied to the distribution of the biomarker at baseline. Out of 512 possible models, the best model based on AIC was selected and a multiple linear regression analysis with Type-III sums of squares was performed using this model for each dose of tirzepatide, placebo and dulaglutide.

**Additional References**

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**Supplemental Table 1. Patient Demographics and Clinical Characteristics at Baseline**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Variables** | **Placebo**  **N=51** | **Tirzepatide 1 mg**  **N=52** | **Tirzepatide 5 mg**  **N=55** | **Tirzepatide 10 mg**  **N=51** | **Tirzepatide 15 mg**  **N=53** | **Dulaglutide 1.5 mg**  **N=54** |
| **Patient Demographics** | | | | | | |
| Age, years | 56.6 (8.9) | 57.4 (8.9) | 57.9 (8.2) | 56.5 (9.9) | 56.0 (7.6) | 58.7 (7.8) |
| Sex, n (%) |  |  |  |  |  |  |
| Women | 22 (43) | 23 (44) | 21 (38) | 21 (41) | 31 (59) | 30 (56) |
| Men | 29 (57) | 29 (56) | 34 (62) | 30 (59) | 22 (42) | 24 (44) |
| Race, n (%) |  |  |  |  |  |  |
| White | 41 (80) | 42 (81) | 46 (84) | 37 (74) | 43 (81) | 44 (83) |
| Asian | 1 (2) | 0 (0) | 0 (0) | 1 (2) | 1 (2) | 2 (4) |
| Black or African American | 2 (4) | 5 (10) | 6 (11) | 7 (14) | 6 (11) | 4 (8) |
| Ethnicity |  |  |  |  |  |  |
| Hispanic or Latino | 27 (59) | 25 (52) | 22 (49) | 26 (57) | 23 (46) | 19 (41) |
| Not Hispanic or Latino | 19 (41) | 23 (48) | 23 (51) | 20 (44) | 27 (54) | 27 (59) |
| **Clinical Characteristics** | | | | | | |
| HbA1c, % | 8.0 (0.9) | 8.2 (0.9) | 8.2 (1.0) | 8.2 (1.1) | 8.1 (1.1) | 8.1 (1.0) |
| HbA1c, mmol/mol | 63.9 (9.8) | 66.1 (9.8) | 66.1(10.9) | 66.1 (12.0) | 65.0 (12.0) | 65.0 (10.9) |
| Fasting plasma glucose, mg/dL1 | 163.1 (41.4) | 161.1 (40.7) | 168.6 (44.3) | 170.6 (50.3) | 164.8 (48.6) | 178.1 (64.5) |
| Fasting plasma glucose, mmol/L | 9.1 (2.3) | 8.9 (2.3) | 9.4 (2.5) | 9.5 (2.8) | 9.2 (2.7) | 9.9 (3.6) |
| eGFR (BSA CKD-EPI calculation; ml/min/1.73 m2)1 | 95.3 (15.3) | 95.6 (16.8) | 92.2 (17.2) | 93.7 (18.6) | 91.8 (17.9) | 90.7 (17.6) |
| Body weight, kg | 91.5 (23.1) | 93.2 (24.4) | 92.8 (19.0) | 92.7 (19.5) | 89.1 (22.7) | 89.8 (16.9) |
| BMI, kg/m2 | 32.4 (6.0) | 32.9 (6.1) | 32.9 (5.7) | 32.6 (5.8) | 32.2 (6.2) | 32.4 (5.4) |
| Diabetes duration, years | 8.6 (7.0) | 7.8 (5.4) | 8.9 (5.7) | 7.9 (5.8) | 8.5 (6.1) | 9.3 (7.1) |
| Metformin, yes, n (%) | 47 (92.2) | 46 (88.5) | 49 (89.1) | 44 (86.3) | 51 (96.2) | 48 (88.9) |

All values are mean (SD) or n (proportions [%]), unless specified. Triglyceride, ApoB and ApoC-III data are presented as antilog. 1All post-baseline data, safety population. The mITT population was used for the rest of the variables. Abbreviations: CKD-EPI=chronic kidney disease epidemiology collaboration. BSA CKD-EPI=Body surface area–adjusted chronic kidney disease epidemiology; eGFR=estimated glomerular filtration rate; HbA1c=glycated hemoglobin A1c. Data published in Frias et al. Lancet. 2018;392(10160):2180-2193.

**Supplemental Table S2. Mean Concentrations at Baseline and Change from Baseline at 26 Weeks**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| NASH-related Biomarker | | Placebo  N=51 | | Tirzepatide 1 mg  N=52 | | Tirzepatide 5 mg  N=55 | | Tirzepatide 10 mg  N=51 | | Tirzepatide 15 mg  N=53 | | Dulaglutide 1.5 mg  N=54 | |
|  |  | meana | p-  value | meana | p-value | meana | p-  value | meana | p-  value | meana | p-  value | meana | p-value |
| ALT, U/L | BL | 26.5 (4.7) |  | 31.0 (4.6) |  | 29.1 (4.5) |  | 38.6 (4.7) |  | 28.1 (4.7) |  | 30.3 (4.6) |  |
| CFB | -6.6 (1.9) | <0.001 | -4.7 (1.8) | 0.010 | -8.4 (1.8) | <0.001 | -11.4 (1.8) | <0.001 | -11.0 (2.0) | <0.001 | -4.6 (1.8) | 0.010 |
| vs PBO | --- | --- | 1.9 (-3.3, 7.0) | 0.479 | -1.8 (-6.9, 3.2) | 0.482 | -4.8 (-10.0, 0.3) | 0.067 | -4.5 (-9.9, 1.0) | 0.107 | 2.0 (-3.1, 7.0) | 0.448 |
| vs DU | --- | --- | -0.1 (-5.1, 4.9) | 0.967 | -3.8 (-8.7, 1.1) | 0.131 | -6.8 (-11.8, -1.8) | 0.008 | -6.4 (-11.7, -1.1) | 0.018 | --- | --- |
| AST, U/L | BL | 22.5 (4.4) |  | 25.4 (4.3) |  | 22.7 (4.3) |  | 33.8 (4.4) |  | 22.6 (4.5) |  | 23.3 (4.3) |  |
| CFB | -5.4 (2.2) | 0.014 | -4.5 (2.1) | 0.033 | -5.5 (2.0) | 0.007 | -3.2 (2.1) | 0.131 | -7.4 (2.3) | 0.002 | -4.7 (2.0) | 0.022 |
| vs PBO | --- | --- | 0.8 (-5.1, 6.8) | 0.780 | -0.1 (-6.0, 5.7) | 0.961 | 2.2 (-3.7, 8.2) | 0.465 | -2.1 (-8.4, 4.2) | 0.516 | 0.7 (-5.1, 6.6) | 0.808 |
| vs DU | --- | --- | 0.1 (-5.6, 5.9) | 0.965 | -0.9 (-6.5, 4.8) | 0.762 | 1.5 (-4.2, 7.2) | 0.609 | -2.8 (-8.9, 3.3) | 0.366 | --- | --- |
| K-18, U/L | BL | 394.4 (50.3) |  | 363.3 (51.9) |  | 375.8 (49.3) |  | 409.9 (50.3) |  | 376.2 (55.0) |  | 478.3 (49.8) |  |
| CFB | -22.6 (37.3) | 0.545 | -55.6 (37.0) | 0.134 | -87.6 (35.9) | 0.015 | -157.8 (37.2) | <0.001 | -110.6 (41.2) | 0.008 | -66.9 (35.1) | 0.058 |
| vs PBO | --- | --- | -32.9 (-136.4, 70.6) | 0.531 | -65.0 (-167.0, 37.0) | 0.211 | -135.2 (-239.0, -31.3) | 0.011 | -88.0 (-197.5, 21.6) | 0.115 | -44.3 (-145.2, 56.7) | 0.388 |
| vs DU | --- | --- | 11.3 (-89.3, 111.9) | 0.824 | -20.7 (-119.7, 78.3) | 0.681 | -90.9 (-191.7, 9.9) | 0.077 | -43.7 (-150.5, 63.1) | 0.421 | --- | --- |
| ProC3, ng/mL | BL | 9.3 (0.6) |  | 9.3 (0.6) |  | 8.7 (0.6) |  | 9.9 (0.6) |  | 8.6 (0.6) |  | 8.8 (0.6) |  |
| CFB | 0.9 (0.5) | 0.077 | -0.5 (0.5) | 0.392 | -0.1 (0.5) | 0.853 | -0.5 (0.5) | 0.325 | -1.2 (0.6) | 0.041 | -0.3 (0.5) | 0.605 |
| vs PBO | --- | --- | -1.4 (-2.8, 0.1) | 0.064 | -1.0 (-2.4, 0.4) | 0.159 | -1.4 (-2.9, 0.0) | 0.051 | -2.1 (-3.6, -0.6) | 0.007 | -1.2 (-2.6, 0.2) | 0.101 |
| vs DU | --- | --- | -0.2 (-1.6, 1.2) | 0.784 | 0.2 (-1.2, 1.5) | 0.817 | -0.2 (-1.6, 1.1) | 0.726 | -0.9 (-2.4, 0.6) | 0.226 | --- | --- |
| Total Adiponectin, mg/L | BL | 4.0 (0.4) |  | 5.4 (0.4) |  | 4.2 (0.4) |  | 4.3 (0.4) |  | 5.1 (0.5) |  | 4.5 (0.41) |  |
| CFB | -0.1 (0.2) | 0.799 | -0.1 (0.2) | 0.718 | 0.4 (0.2) | 0.090 | 0.9 (0.2) | <0.001 | 0.9 (0.3) | <0.001 | 0.3 (0.2) | 0.103 |
| vs PBO | --- | --- | -0.0 (-0.6, 0.6) | 0.941 | 0.4 (-0.2, 1.0) | 0.174 | 0.9 (0.3, 1.5) | 0.003 | 1.0 (0.3, 1.6) | 0.004 | 0.4 (-0.2, 1.0) | 0.189 |
| vs DU | --- | --- | -0.4 (-0.1, 0.2) | 0.163 | 0.0 (-0.6, 0.6) | 0.962 | 0.5 (-0.1, 1.1) | 0.086 | 0.6 (-0.1, 1.2) | 0.083 | --- | --- |

aBaseline and change from baseline at Week 26 data are presented as LSM (SE) and treatment differences (vs PBO and vs DU) are presented as LSM (95% confidence interval); p-values for within-treatment CFB and between-treatment (vs PBO and vs DU) are shown. Abbreviations: ALT=alanine aminotransferase; AST=aspartate aminotransferase; BL=baseline; CFB=change from baseline; DU=dulaglutide; K-18=keratin-18; LSM=least squares mean; N=number of patients included in the modified intent-to-treat population; PBO=placebo; SE=standard error.

Coefficients of variation across treatment groups for the baseline values of the biomarkers were as follows: ALT (13.83%); AST (17.65%); K-18 (10.47%); Pro-C3 (5.43%); total adiponectin (11.98%).

**Supplemental Table S3. Contribution of Relevant Confounding Variables to the Variability of the Change from Baseline in Log ALT**1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Important Variable | Percent of Explained Variability | | | | | |
| Placebo | Tirzepatide 1 mg | Tirzepatide 5 mg | Tirzepatide 10 mg | Tirzepatide 15 mg | Dulaglutide 1.5 mg |
| Baseline Log ALT | 1.03 | 29.82 | 30.41 | 62.38 | 29.39 | 41.51 |
| CFB Weight | 5.65 | 11.95 | 21.46 | 4.61 | 15.3 | 4.22 |
| Baseline Weight | 8.94 | 3.55 | 2.58 | 0.15 | 10.5 | 1.55 |

Data presented as %. 1ALT was log-transformed for this analysis due to skewness of the data. CFB=change from baseline

**Supplemental Table S4. Contribution of Relevant Confounding Variables to the Variability of the Change from Baseline in Log AST**1

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Important Variable | Percent of Explained Variability | | | | | |
| Placebo | Tirzepatide 1 mg | Tirzepatide 5 mg | Tirzepatide 10 mg | Tirzepatide 15 mg | Dulaglutide 1.5 mg |
| Baseline Log AST | 15.46 | 64.25 | 32.63 | 58.44 | 40.22 | 41.89 |
| CFB Weight | 3.63 | 4.77 | 5.85 | 0.18 | 10.9 | 0.29 |
| Baseline Weight | 2.01 | 4.66 | 0.34 | 0.11 | 2.77 | 0.72 |
| CFB Log Triglycerides | 0.24 | 9.75 | 2.04 | 0.09 | 0.25 | 0.51 |

1AST was log-transformed for this analysis due to skewness of the data. CFB=change from baseline

**Supplemental Table S5. Contribution of Relevant Confounding Variables to the Variability of the Change from Baseline in Log K-181**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Important Variable | Percent of Explained Variability | | | | | |
| Placebo | Tirzepatide 1 mg | Tirzepatide 5 mg | Tirzepatide 10 mg | Tirzepatide 15 mg | Dulaglutide 1.5 mg |
| Baseline Log K-18 | 18.87 | 38.27 | 24.44 | 64.29 | 31.69 | 32.95 |
| CFB HbA1c | 0.11 | 5.59 | 5.33 | 0 | 7.48 | 0.13 |
| Baseline HbA1c | 2.26 | 6.76 | 0.46 | 0.01 | 2.94 | 0.53 |

1K-18 was log-transformed for this analysis due to skewness of the data. CFB=change from baseline

**Supplemental Table S6. Contribution of Relevant Confounding Variables to the Variability of the Change from Baseline in Pro-C3**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Important Variable | Percent of Explained Variability | | | | | |
| Placebo | Tirzepatide 1 mg | Tirzepatide 5 mg | Tirzepatide 10 mg | Tirzepatide 15 mg | Dulaglutide 1.5 mg |
| Baseline Pro-C3 | 2.12 | 5.48 | 28.68 | 34.83 | 47.91 | 42.9 |
| CFB Weight | 1.92 | 1.02 | 2.58 | 7.1 | 5.32 | 9.42 |
| CFB HbA1c | 0.09 | 0.90 | 3.4 | 0.68 | 0.13 | 0.33 |
| CFB Log Fasting Insulin | 5.37 | 0.06 | 1.34 | 0.37 | 7.15 | 1.34 |
| Age | 0.13 | 1.89 | 1.11 | 2.4 | 0.03 | 0.05 |

CFB=change from baseline

**Supplemental Table S7. Contribution of Relevant Confounding Variables to the Variability of the Change from Baseline in Log Total Adiponectin1**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Important Variable | Percent of Explained Variability | | | | | |
| Placebo | Tirzepatide 1 mg | Tirzepatide 5 mg | Tirzepatide 10 mg | Tirzepatide 15 mg | Dulaglutide 1.5 mg |
| Baseline Log Total Adiponectin | 19.94 | 7.00 | 4.24 | 4.94 | 17.21 | 7.78 |
| CFB Weight | 0.03 | 10.28 | 3.42 | 25.92 | 0.41 | 5.94 |
| Baseline Weight | 5.30 | 9.72 | 2.15 | 8.18 | 6.81 | 2.08 |

1Total Adiponectin was log-transformed for this analysis due to skewness of the data.