Impact on glycaemic variability after treatment with dapagliflozin as dual therapy with metformin on Mexican type 2 diabetes patients. A randomized, open-label study.

1. Project Title
Impact on glycaemic variability after treatment with dapagliflozin as dual therapy with metformin on Mexican type 2 diabetes patients. A randomized, open-label study

2. Protocol Version
Version 2, December 4th, 2018

3. Type of Investigation

<table>
<thead>
<tr>
<th>Type of investigation</th>
<th>Select an option</th>
</tr>
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<tbody>
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<td>Pharmacologic</td>
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<td>Interchangeability</td>
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4. Investigators

<table>
<thead>
<tr>
<th>INVESTIGATOR</th>
<th>Institucional Position</th>
<th>Position in the project</th>
<th>Telephone(ex t.)</th>
<th>e-mail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miguel Ángel Gómez Sámano</td>
<td>Specialist physician A</td>
<td>Principal Investigator</td>
<td>+52 55 5487 0900 EXT. 2405</td>
<td><a href="mailto:gsamano83@yahoo.com">gsamano83@yahoo.com</a></td>
</tr>
<tr>
<td>Daniel Cuevas Ramos</td>
<td>Specialist physician A</td>
<td>Associate Investigator</td>
<td>+52 55 5487 0900 EXT. 2405</td>
<td><a href="mailto:ceptamim@gmail.com">ceptamim@gmail.com</a></td>
</tr>
<tr>
<td>Francisco J Gómez Pérez</td>
<td>Chief Endocrinology Department</td>
<td>Associate Investigator</td>
<td>+52 55 5487 0900 EXT. 2405</td>
<td><a href="mailto:gomezperezfco@gmail.com">gomezperezfco@gmail.com</a></td>
</tr>
<tr>
<td>Alberto Sigfrido Benítez Rentería</td>
<td>MSL Az</td>
<td>Associate Investigator</td>
<td>+5215561663 977</td>
<td><a href="mailto:sigfridomed@gmail.com">sigfridomed@gmail.com</a></td>
</tr>
<tr>
<td>Alejandra Silva Giordano</td>
<td>Medical Manager AZ</td>
<td>Associate Investigator</td>
<td>+5215561663 977</td>
<td><a href="mailto:alejandra.silva@astrazeneca.com">alejandra.silva@astrazeneca.com</a></td>
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5. Participant Institutions

<table>
<thead>
<tr>
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<th>Did the institution accepted the protocol?</th>
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<tr>
<td>Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán</td>
<td>Yes</td>
</tr>
<tr>
<td>Vasco de Quiroga 15 Belisario Domínguez, Sección XVI, 14080 Mexico City, México</td>
<td></td>
</tr>
</tbody>
</table>

6. Grant

6a. Sponsor

Astra Zeneca México will provide the resources defined in the contract to acquire investigational drug, laboratory data, medical writing, cost of publication and glucose lowering sensors.

6b. Specify if investigators will receive payment for their participation in the protocol.

No

7. Summary

Glycaemic variability is referred as swings in glucemic concentration throughout the day, including preprandial and postprandial glucose, and it has been proposed that could be determinant in the development of microvascular complications of type 2 diabetes (Brownlee and Hirsch 2006).

SGLT2 inhibitors (SGLT2i) are a novel group of medications for treating type 2 diabetes patients but their effect on glucose variability has not been determined as a primary endpoint in clinical trials of type 2 diabetes mellitus patients. The aim of this study is to compare the effect of SGLT2 inhibition on glucose variability on new onset type 2 DM patients. Methods: We will include 88 patients with type 2 diabetes diagnosis with an Hba1c ≥ 7.5% and ≤ 9%, with BMI > 25 and <45 kg/m², drug-naïve subjects.

There will be a pre-randomization run-in period in which subjects will receive metformin 500mg twice daily for two weeks and then patients that tolerate this dose will be randomized 1:1 to receive a daily dosage of dapagliflozin 10 mg and 2000 mg metformin for 12 weeks (n=40) or 2000 mg metformin (n=40). Patients who do not tolerate metformin at 2000mg dose will be down titrated to 1500 mg daily, patients who do not tolerate metformin at 1500mg daily, will be down titrated to 1000 daily. In case patients do not tolerate 1000 mg daily, they will be excluded.

Both groups will be monitored for 7 days using either iPro™ CGM system (Medtronic, Northridge, CA) or Dexcom G6 CGM (Dexcom Inc, San Diego, CA). Basal continuous glucose monitoring will start at week 1 (first visit), and removed at day 7 and final continuous glucose monitoring will start at week 11 and removed 7 days after (final visit).

The main variables of interest are going to be in order of importance the delta of change (before and after study entry) of: 1.- glycaemic variability, 2.- Change in insulin level concentrations 3.- change in Hba1c. 4.- change in weight, 5.- Blood pressure and 6.- waist circumference; before and after SGLT2i. The expected results are: compared to standard treatment, dapagliflozin arm will have lower glycaemic variability, higher reduction in Hba1c and lower blood pressure.

8. Background
Glycaemic variability is referred as swings in glucemic concentration throughout the day, including preprandial and postprandial glucose, and it has been proposed that could be determinant in the development in microvascular complications of type 2 diabetes (Brownlee and Hirsch 2006). SGLT2 inhibitors (SGLT2i) are a novel group of medications for treating type 2 diabetes patients but their effect on glucose variability has not been determined as a primary endpoint in clinical trials of type 2 diabetes mellitus patients.

9. Problem definition
There is few scientific evidence in regards dapagliflozin effect in glycaemic variability in patients with type 2 diabetes

10. Justification
Patients with type 2 diabetes regularly have either overweight and obesity, and hypertension. In patients with these characteristics, dapagliflozin is often used as adjuvant treatment. This therapeutic decision is supported by international diabetes guidelines.

11. Hypothesis
Compared to standard treatment, dapagliflozin arm will have lower glycaemic variability, higher reduction in Hba1c and lower blood pressure.

12. Objectives.
Primary objective:
To describe the effect of dapagliflozin therapy on glycaemic variability in type 2 diabetes patients.
Outcome measure:
Mean difference of glycaemic variability (MAGE) in mmol/L

Secondary objectives
1. To describe the effect of dapagliflozin on insulin serum concentration, Hba1c, on weight, blood pressure, waist circumference after 12 weeks of treatment on subjects with type 2 diabetes

Outcome measures:
- Mean difference insulin serum concentrations µU/mL
- Mean difference of %HbA1c
- Mean difference in kilograms
- Mean difference in mmHg
- Mean difference waist measured in centimeters

The aim of this study is to compare the effect of SGLT2 inhibition on glucose variability on new onset type 2 DM patients. Methods: We will include 88 patients with type 2 diabetes diagnosis with an Hba1c ≥ 7.5% and ≤ 9%, with BMI > 25 and <45 kg/m², drug-naïve subjects. There will be a pre-randomization run-in period in which subjects will receive metformin 500mg twice daily for two weeks and then patients that tolerate this dose will be randomized 1:1 to either receive a daily
dosage of dapagliflozin 10 mg and 2000 mg metformin for 12 weeks (n=44) or 2000 mg metformin (n=44). Patients who do not tolerate metformin at 2000mg dose will be downtitrated to 1500 mg daily, patients who do not tolerate metformin at 1500mg daily, will be dowtitrated to 1000 daily. In case patients do not tolerate 1000 mg daily, they will be excluded. Patients who do not achieve glycaemic control, another antihyperglycaemic drug can be used (See section 20, rescue therapy).

Both groups will be monitored for 7 days using either iPro™ CGM system (Medtronic, Northridge, CA) or Dexcom G6 CGM (Dexcom Inc, San Diego, CA), or other available in Mexico. Basal continuous glucose monitoring will start at week 1 (first visit), and removed at day 7 and final continuous glucose monitoring will start at week 11 and removed 7 days after (final visit).

The main variables of interest are going to be in order of importance the delta of change (before and after study entry) of: 1.- glycaemic variability, 2.- Change in insulin level concentrations 3.- change in Hba1c. 4.- change in weight, 5.- Blood pressure and 6.- waist circumference; before and after SGLT2i. The expected results are: compared to standard treatment, dapagliflozin arm will have lower glycaemic variability, higher reduction in Hba1c and lower blood pressure.
Assessed for eligibility

2-week Run-in Period & metformin titration

Not-included (n=8)
- Not meeting inclusion criteria
- Declined to participate
- Do not tolerate metformin

Enrollment visit 1 (n=88)
Clinical history
Physical exam
Basal lab tests
Glucose monitoring system (7 days)

Visit 2: Week 1
Glucose monitoring system removal (7 days)

Visit 3: Week 4
Physical exam
Follow-up lab tests

Visit 4: Week 8
Physical exam
Follow-up lab tests

Visit 5: Week 12
Physical exam
lab tests
Glucose monitoring system (7 days)

Visit 6: Week 12
Glucose monitoring system removal (7 days)
### 17. Treatments (if applicable)

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<thead>
<tr>
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<th>Information</th>
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<th>NA</th>
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<td><strong>Name</strong></td>
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</tr>
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<td>Does it comply with &quot;Good manufacturing practices&quot;?</td>
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<tr>
<td>Codes, labeling, storage, retention and protection of drug samples</td>
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<tr>
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<tr>
<td><strong>Velocity of administration</strong></td>
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<td><strong>Duration of treatment</strong></td>
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<td><strong>Pharmaceutical form</strong></td>
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<tr>
<td><strong>Velocity of administration</strong></td>
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<tr>
<td><strong>Duration of treatment</strong></td>
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<tr>
<td><strong>Number of visits and Schedule</strong></td>
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<td><strong>Duration of each phase of the study</strong></td>
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<td><strong>Lab tests that will be used.</strong></td>
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<td><strong>Total duration of follow-up</strong></td>
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<td><strong>Methods Métodos de muestreo</strong></td>
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<tr>
<td><strong>Treatment options that will be offered at the end of the study.</strong></td>
<td>Metformin and standard of care</td>
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</tbody>
</table>

### 19. Overdose management.

If a Patient for accident ingests more tablets than recommended, blood pressure, glucose will be assessed closely every 4 hours. Dapagliflozin, due to its mechanism of action, do not cause hypoglycemia.

### 20. Rescue therapy
Group 1: Dapagliflozin 10 mg/day + metformin 2000 mg/day: glimepiride, glibenclamide, DPP4i (1st line) or insulin (2nd line).
Group 2: Metformin 2000 mg/day: glimepiride, glibenclamide, DPP4i (1st line) or insulin (2nd line).

21. Concomitant therapies allowed.

Group 1 (Dapagliflozin + metformin): NSAIDs, acetaminophen, antibiotics, statin, ezetimibe, ACE/ARB2 inhibitors, Serotonin recapture inhibitors, bezafibrate, amlodipine, b-blocker, and other pharmacologic groups that according to investigators does not interfere with the protocol.

Group 2 (metformin): NSAIDs, acetaminophen, antibiotics, statin, ezetimibe, ACE/ARB2 inhibitors, Serotonin recapture inhibitors, bezafibrate, amlodipine, b-blocker, and other pharmacologic groups that according to investigators does not interfere with the protocol.

22. Concomitant therapies prohibited.

Group 1 (Dapagliflozin + metformin): GLP-1 RA, corticosteroids, other SGLT2i.
Group 2 (metformin): GLP-1 RA, corticosteroids, other SGLT2i.

23. Definition of the monitoring variables

The main variables of interest are going to be in order of importance the delta of change (before and after study entry) of:
1. - glycaemic variability, 2. - Change in insulin level concentrations 3. - change in Hba1c. 4. - change in weight, 5. - Blood pressure and 6. - waist circumference; before and after SGLT2i. The expected results are: compared to standard treatment, dapagliflozin arm will have lower glycaemic variability, higher reduction in Hba1c and lower blood pressure.

Outcome measure for main variable:
Mean difference of glycaemic variability (MAGE) in mmol/L

Outcome measure for other variables:
- Mean difference insulin serum concentrations µU/mL
- Mean difference of HbA1c (in %)
- Mean difference in kilograms
- Mean difference in mmHg
- Mean difference waist measured in centimeters

24. Methods that will be used to collect information

A physical data capture sheet will be made, only the initials of patients and the internal number of the protocol will be saved.

25. Criteria of failure and success

Fail: There is no decrease in glycaemic variability with the use of dapagliflozin
Success: There exists decrease in glycaemic variability with the use of dapagliflozin

26. Sample size

Sample size was determined using t distribution:

\[ n = \left( Z_{\alpha/2} + Z_{\beta} \right)^2 \times 2 \times \sigma^2 / d^2, \]

Where \( Z_{\alpha/2} \) is the critical value of the Normal distribution at \( \alpha/2 \) (e.g. for a confidence level of 95%, \( \alpha \) is 0.05 and the critical value is 1.96), \( Z_{\beta} \) is the critical value of the Normal distribution at \( \beta \) (e.g. for a power of 80%, \( \beta \) is 0.2 and the critical value is 0.84), \( \sigma^2 \) is the population variance, and \( d \) is the difference you would like to detect.

We substitute the critical values of the Z distribution to the T distribution using 71 degrees of freedom.
1.99/2 critical value p 0.05 of α
Critical value 0.20 (β) = 1.29

\[ N = \frac{(0.995+1.29)^2 * 2 * 30}{15.3^2} \]

\[ N = 5.221225 \times 2 \times 900 / 234.09 \]

N= 40 per group

Total 80 subjects + considering 10% of loss = 88 subjects

27. Description of techniques, instruments and appliances that will be used in measurements

Blood pressure will be measured considering the mean of two determinations after seating for at least 5 minutes. Mean arterial pressure (MAP) will be calculated with the formula: (systolic blood pressure * 0.33) + (diastolic blood pressure * 0.66). Anthropometric variables will be measured using standardized techniques; these included height, weight, waist and hip circumferences, and quantification of body fat using bioelectrical impedance with the Jawon scale model IOI 353 - JMW160. BMI will be calculated using the standard equation, weight/height² (kg/m²). After at least eight-hour fasting, glucose, lipid profile (total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides), creatinine, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albuminuria in a 24 h urine collection will be measured using an automated International Journal of Nephrology 3 analyzer (Synchon CX Beckman, Fullerton CA). LDL cholesterol (LDL) will be estimated by the Friedewald equation (LDL= total cholesterol - HDL - triglycerides /5)[23]. Glycated hemoglobin (A1c) was measured using HPLC (Variant II Turbo, Biorad), certified by the National Glycohemoglobin Standardization Program (NGSP). Creatinine clearance will be calculated using the CKD-EPI equation. All samples will be measured in the Laboratorio Central of Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran (http://www.innsz.mx/opencms/contenido/departamentos/labcentral/)

Insulin levels will be measured using ELISA Kits (Merck Millipore, USA)

100% of the samples are certified by the College of American Pathologists (71893-07-01)

28. Description of evaluation sheets, questionnaires, etc, that will be used.

We will use the traditional medical history data sheet. An exercise questionnaire will be used (GPAQ questionnaire, World Health Organization: www.who.int/chp/steps) and a 7-days homemade food registry.

29. Does the protocol involve the handling and labeling of biological samples? If applicable, mention the procedures that will be used.

Yes, with patients initials.

30. Corresponding information to ensure that the biological samples obtained will not be used for permanent or immortal cell lines or purposes unrelated to the study.

N/A

31. Description of treatment groups.

We will include 88 patients with type 2 diabetes diagnosis with an Hba1c ≥ 7.5% and ≤ 9%, with BMI > 25 and <45 kg/m², drug-naïve subjects. Subjects enrolled will be randomized 1:1 to either receive a daily dosage of dapagliflozin 10 mg and 2000 mg metformin for 12 weeks (n=18) or 2000 mg metformin (n=18). Patients who do not tolerate metformin at 2000mg dose will be downtitrated to 1500 mg daily, patients who do not tolerate metformin at 1500mg daily, will be down titrated to 1000 mg daily. In case patients do not tolerate 1000 mg daily, they will be excluded.

Both groups will be monitored for 7 days using either iPro™ CGM system (Medtronic, Northridge, CA) or Dexcom G6 CGM (Dexcom Inc, San Diego, CA). Basal continuous glucose monitoring will start at week 1 (first visit), and removed at day 7 and final continuous glucose monitoring will start at week 11 and removed 7 days after (final visit).
A Randomization Plan
from
http://www.randomization.com

1. metformina + tratamiento estándar
2. Dapagliflozina + metformina
3. metformina + tratamiento estándar
4. Dapagliflozina + metformina
5. metformina + tratamiento estándar
6. metformina + tratamiento estándar
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39. Dapagliflozina + metformina
40. metformina + tratamiento estándar
41. metformina + tratamiento estándar
42. metformina + tratamiento estándar
88 subjects randomized into 22 blocks
To reproduce this plan, use the seed 9476
along with the number of subjects per block/number of blocks
and (case-sensitive) treatment labels as entered originally.
Randomization plan created on 22/5/2019 13:46:39

33. If you include a placebo group, include the justification below.
NA

34. Criteria for the premature withdrawal of the study
Allergie due to Dapagliflozin, severe illness that compromise life, pregnancy during the study. Patients who present acute kidney injury (decrease in >40% of GFR) Patients that do not tolerate at least 1000 mg /day metformin.

35. Procedures for the removal of a patient from the study
The patient will be notified of the withdrawal from the study and will be given the standard treatment of any patient with type 2 diabetes mellitus.

36. Criteria for the premature (partial or complete) suspension of the study
N/A

37. Selection criteria
a) Inclusion criteria
- Subjects > 18-77 years-old
- Both Male and female
- Hba1c ≥ 7.5 % and ≤9%
- BMI > 25 and <45 kg/m²
- Type 2 diabetes diagnosis, drug-naïve

b) Exclusion criteria
- Hba1c > 9%
- Creatinine clearance CKD-EPI: < 60 mL/min
- LADA or Type 1 diabetes
- Gestational diabetes
- Clinically significant disease like: hepatic, hematological, oncological, psychiatric or rheumatic disease.
- Symptoms of marked uncontrolled diabetes: (marked polyuria or polydipsia + 10% weight loss prior the last 3 months enrolment)
- Known hypersensitivity to dapagliflozin or any of the excipients of the product
- eGFR persistently <45 mL/min/1.73 m²
- Unstable or rapidly progressing renal disease
- Patients with severe hepatic impairment (Child-Pugh class C)
- Any major CV event/Vascular Disease within 3 months prior to signing the consent at enrolment, as assessed by the investigator
- For women only - currently pregnant (confirmed with positive pregnancy test) or breast-feeding.

c) Elimination criteria
- Allergie due to Dapagliflozin, severe illness that compromise life, pregnancy during the study. Patients who present acute kidney injury (decrease in >40% of GFR). Patients that do not tolerate at least 1000 mg /day metformin.

- For women only – pregnancy confirmed with serum chorionic gonadotropin. In case of pregnancy during the study, the subject has to be eliminated from the trial, will be offered standard treatment and Pregnancy Outcome Report (Annex) has to be filled to record the mother’s essential details. Part I should be filled in after the pregnancy has been identified and Part II is to record the outcome of the pregnancy.
38. Outcome and variables

The main variables of interest are going to be in order of importance the delta of change (before and after study entry) of: 1.- glycaemic variability, 2.- Change in insulin level concentrations 3.- change in Hba1c. 4.- change in weight, 5.- Blood pressure and 6.- waist circumference; before and after SGLT2i. The expected results are: compared to standard treatment, dapagliflozin arm will have lower glycaemic variability, higher reduction in Hba1c and lower blood pressure.

Outcome measure for main variable:
Mean difference of glycaemic variability (MAGE) in mmol/L

Outcome measure for other variables:
- Mean difference insulin serum concentrations µU/mL
- Mean difference of HbA1c in %
- Mean difference in kilograms (weight)
- Mean difference in mmHg (Blood pressure)
- Mean difference waist measured in centimeters

39. Methods that will be used to contact patients

Personal data will be obtained for contact as phone number, mobile phone number, email through the data collection form.

40. Statistical analysis plan.

Normality of variables are going to be analyzed using Kolmogorov-Smirnov test. According to the distribution of variables, they are going to be expressed as mean and standard deviation (DE) or median and interquartile range (IQR) for variables with normal or biased distribution, respectively. Glucose variability is going to be determined using the MAGE and CONGA indexes. Comparison between the two groups is going to be evaluated using ANCOVA. A p value based on two-sided tests ≤0.05 is going to be considered significant. All analyses are going to be performed with SPSS 20.0 (Chicago, IL).

41. Sample size justification

Expecting a mean difference of 20 mg/dL (1.1 mmol/L) in the MAGE index with and standard deviation of 30 mg/dL (1.7 mmol/L), with an alpha value of 0.05 and a power of 0.80, 40 subjects in each group will be needed (+10% for losses) \( \Rightarrow 88 \)

Sample size was determined using t distribution:

\[ n = \left(\frac{Z_{\alpha/2} + Z_{\beta}}{\sigma}\right)^2 \times \frac{2 \times \sigma^2}{d^2}, \]
Where $Z_{α/2}$ is the critical value of the Normal distribution at $α/2$ (e.g. for a confidence level of 95%, $α$ is 0.05 and the critical value is 1.96), $Z_β$ is the critical value of the Normal distribution at $β$ (e.g. for a power of 80%, $β$ is 0.2 and the critical value is 0.84), $σ^2$ is the population variance, and $d$ is the difference you would like to detect. We substitute the critical values of the Z distribution to the T distribution using 71 degrees of freedom.

$T_{α/2}$

1.99/2 critical value is 0.05 of $α$

Critical value $0.20 (β) = 1.29$

$N = \frac{(0.995 + 1.29)^2 \times 2 \times 302}{15.32}$

$N = \frac{5.221225 \times 2 \times 900}{234.09}$

$N = 40 \text{ per group}$

Total 80 subjects + considering 10% of loss = 88 subjects


42. Subject expected to be enrolled

88 subjects

43. In case of multicenter study, include global number and local number of sample

N/A

44. Procedures for reporting a deviation from original statistical plan

<table>
<thead>
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<th>Secondary end points</th>
<th>Overall population</th>
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<th>Insulin statin</th>
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<td>Dapagliflozin</td>
<td>Placebo</td>
<td>Dapagliflozin</td>
</tr>
<tr>
<td>Furosamide, mg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline mean (SD)</td>
<td>293.5 (6.7)</td>
<td>307.9 (7.3)</td>
<td>279.3 (8.9)</td>
</tr>
<tr>
<td>Adjusted mean (SE)</td>
<td>-20.4 (3.2)</td>
<td>-9.6 (3.2)</td>
<td>-20.2 (4.9)</td>
</tr>
<tr>
<td>$P$ value for treatment difference</td>
<td>0.019</td>
<td>0.012</td>
<td>0.012</td>
</tr>
<tr>
<td>24-h MAGE, mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline mean (SD)</td>
<td>102.7 (30.9)</td>
<td>108.6 (29.2)</td>
<td>89.3 (28.8)</td>
</tr>
<tr>
<td>Adjusted mean (SE)</td>
<td>-10.0 (4.1)</td>
<td>+5.3 (4.1)</td>
<td>-7.3 (6.2)</td>
</tr>
<tr>
<td>$P$ value for treatment difference</td>
<td>0.010</td>
<td>0.110</td>
<td>0.060</td>
</tr>
<tr>
<td>“Distance traveled” mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline mean (SD)</td>
<td>793.9 (267.5)</td>
<td>779.7 (167.0)</td>
<td>781.9 (338.9)</td>
</tr>
<tr>
<td>Adjusted mean (SE)</td>
<td>-28.0 (26.3)</td>
<td>+9.5 (25.9)</td>
<td>-50.0 (39.5)</td>
</tr>
<tr>
<td>$P$ value for treatment difference</td>
<td>0.312</td>
<td>0.357</td>
<td>0.597</td>
</tr>
<tr>
<td>SD of 24-h glucose, mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline mean (SD)</td>
<td>42.9 (13.1)</td>
<td>43.9 (11.9)</td>
<td>36.7 (10.7)</td>
</tr>
<tr>
<td>Adjusted mean (SE)</td>
<td>-3.4 (1.6)</td>
<td>+1.1 (1.6)</td>
<td>-1.6 (2.4)</td>
</tr>
<tr>
<td>$P$ value for treatment difference</td>
<td>0.037</td>
<td>0.051</td>
<td>0.031</td>
</tr>
</tbody>
</table>

Study was powered only for the overall population; although $P$ values are supplied for the individual statins, inferences for treatment differences should not be made.

MAGE: mean amplitude of glucose excursion; SE, standard error.

Result

$\frac{|M_1 - M_2|}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} = 20.3, 95\% \text{ CI} [8.353, 32.207]$.

You can be 95% confident that the difference between your two population means ($μ_1 - μ_2$) lies between and 32.207.
A letter from the research committee will be sent for approval.

**45. Possible discomforts resulting from the study**

Attending follow-up visits, blood samples.

**46. Potential risks**

Inherent in blood sample.

**47. Anticipated risk detection methods**

Patients will be asked to report, 24 hours a day, the appearance of an adverse effect such as nausea, vomiting, diarrhea, fever or dysuria, or any other.

Adverse events will be recorded and managed prospectively according to local legislation (NOM 220 SSA1 2016) and standardized adverse reaction forms, in case of a Serious Adverse Event, the subject will be referred to the emergency department of the Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran and treated accordingly to local procedures and PV Legislation in addition, all SAEs should be submitted to the AstraZeneca Product Safety mailbox, AEmailboxClinicalTrialTCS@astrazeneca.com, and patientSafety.mexico@astrazeneca.com via “T02 format” (See annex 1) in no more than 24 hours, this submission should include the evidence of submission to the Ministry of Health (MoH).

In case of receiving any pregnancy report during the study, the pregnancy format (Pregnancy outcome report) should be completed for the purpose of documenting the follow-up appropriately during pregnancy and once it has been resolved.

In case to receive any emerging safety issues or unanticipated problems, this should be communicated in no more than 24 hours and at least in parallel with correspondence to regulators, IRB / IEC and Investigators.

The investigator must communicate the initiation of the clinical stage in Mexico (data collection/first visit of the first patient) of the study and a notice of Completion of the clinical stage in Mexico of the study (end of data collection/last visit of the last patient), by means of a submission in a maximum of 15 working days from the beginning or end of the clinical stage in Mexico.

Notice shall be given to the PV National center of cancellation, suspension, discontinuation and/or resumption (including the reasons for this), the document should be performed according to the local pharmacovigilance guidelines. The notice shall be within a maximum of 15 working days after cancellation, suspension and/or discontinuation.

The evidence of submission of the communications must be sent to PatientSafety.Mexico@astrazeneca.com in a period of 48 hours after the submission. If the investigator receives a response from MoH this response should be sent to PatientSafety.Mexico@astrazeneca.com in a period of 48 hours after the document acknowledgment.

Adverse Event: An AE is any untoward medical occurrence in a patient administered a study drug which may or may not have a causal relationship with the study drug. Therefore, an AE is any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease, which is temporally associated with the use of a study drug, whether or not considered related to the study drug. An AE also includes any worsening (ie, any clinically significant change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the study drug.

Serious Adverse Event: A SAE is any untoward medical occurrence that at any dose:

- Results in death – includes all deaths, even those that appear to be completely unrelated to study drug (eg, a car accident in which a patient is a passenger).
- Is life-threatening – in the view of the investigator, the patient is at immediate risk of death at the time of the event. This does not include an AE that had it occurred in a more severe form, might have caused death.
- Requires in-patient hospitalization or prolongation of existing hospitalization. In-patient hospitalization is defined as admission to a hospital or an emergency room for longer than 24 hours. Prolongation of existing hospitalization is
defined as a hospital stay that is longer than was originally anticipated for the event, or is prolonged due to the
development of a new AE as determined by the investigator or treating physician.
Results in persistent or significant disability/incapacity (substantial disruption of one’s ability to conduct normal life
functions).
Is a congenital anomaly/birth defect.
Is an important medical event - Important medical events may not be immediately life-threatening or result in death or
hospitalization, but may jeopardize the patient or may require intervention to prevent 1 of the other serious outcomes
listed above (eg, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or
convulsions that do not result in hospitalization; or development of drug dependency or drug abuse).

48. Security measures for timely diagnosis and prevention of risks
Patients who present a side effect to revision will be cited for assessment.

49. Procedures to be followed to resolve the risks in case they arise
Treatment of the complication or side effect will be given, either by prescription on an outpatient basis or it will be
referred to the service of urgencies to the patient for its assessment and treatment. The emergency expenses will be
borne by the patient since there is no budget to insure the subjects of study. Dapagliflozin is on the market and
approved by FDA, EMA and COFEPRIS.

50. Expected direct benefits
Improvement in glycemic variability

51. Indirect benefits expected
Improvement in weight, pressure, uric acid

52. Overall weighting of risks against benefits of the proposed study
Greater benefits with respect to risks. SGLT2i are recommended as a second line after metformin for its additional
benefits in weight loss, blood pressure and cardiovascular safety, with an acceptable safety profile.

53. Specify costs (direct/indirect, monetary, in time of participation, visits) that the investigation will require from
study subjects
Patients will be required to attend 1 enrollment visit and 6 follow up visits in the next 12 weeks (transportation costs)

54. Specify if the consultations, laboratory / cabinet examinations and medical / surgical treatments generated
during the study will be covered by the patient / research subject
Because there is no budget to insure the patient, the expenses for emergencies or associated complications will be
covered by the subject of the study.

55. Who will cover investigation associate costs.
Astra Zeneca México will provide the resources defined in the contract to acquire investigational drug, laboratory
data, medical writing, cost of publication and glucose lowering sensors.

<table>
<thead>
<tr>
<th>TOOL</th>
<th>Trademark</th>
<th>Presentation</th>
<th>Unit Price</th>
<th>Quantity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>iPro™2 Continuous glucose monitor.</td>
<td>Medtronic®</td>
<td>1</td>
<td>$1,622.00</td>
<td>4</td>
<td>$6,488.00</td>
</tr>
<tr>
<td>Sensors</td>
<td>Medtronic®</td>
<td>10</td>
<td>$378.50</td>
<td>14</td>
<td>$5,299.00</td>
</tr>
<tr>
<td>glucose</td>
<td>immunometric</td>
<td>88 samples</td>
<td>$1,000.00</td>
<td>2</td>
<td>$2,000.00</td>
</tr>
<tr>
<td>insulin</td>
<td>Merck Millipore®</td>
<td>Kit/88 samples ELISA</td>
<td>$1,892.00</td>
<td>2</td>
<td>$3,784.00</td>
</tr>
<tr>
<td>Medical writing</td>
<td></td>
<td></td>
<td>$2,600.00</td>
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<td>$2,600.00</td>
</tr>
<tr>
<td>Cost of publication</td>
<td></td>
<td>$ 4,000.00</td>
<td>1</td>
<td>$4,000.00</td>
<td></td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td>---------------------</td>
<td></td>
</tr>
<tr>
<td>FORXIGA 10mg AstraZeneca</td>
<td>28 tablets</td>
<td>0 (will be covered by local market)</td>
<td>264</td>
<td>$0.00</td>
<td></td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>$24,171.00</strong></td>
<td></td>
</tr>
</tbody>
</table>

56. If applicable, specify the incentives that will be offered (incentive is understood as an offer or influence that compels to perform an action without implicitly a significant deviation with our general plan of life; Gr.: Give a book for having participated)

**Note:** A compensation / incentive outside the proportion is considered a coercive attitude

N/A

57. References.

AACE/ACE Comprehensive Type 2 Diabetes Management Algorithm. 2018

A randomized controlled trial comparing the effects of dapagliflozin and DPP-4 inhibitors on glucose variability and metabolic parameters in patients with type 2 diabetes mellitus on insulin.
Nomoto H, Miyoshi H, Sugawara H, Ono K, Yanagiya S, Oita M, Nakamura A, Atsumi T.

Solini A, Giannini L, Seghieri M, Vitolo E, Taddei S, Ghiadoni L, Bruno RM.

SGLT2-inhibitor and DPP-4 inhibitor improve brain function via attenuating mitochondrial dysfunction, insulin resistance, inflammation, and apoptosis in HFD-induced obese rats.
Sa-Nguanmoo P, Tanakaj P, Kerdphoo S, Jaiwongkam T, Pratchayasakul W, Chattipakorn N, Chattipakorn SC.

Influence of Dapagliflozin on Glycemic Variations in Patients with Newly Diagnosed Type 2 Diabetes Mellitus.
Li FF, Gao G, Li Q, Zhu HH, Su XF, Wu JD, Ye L, Ma JH.

Protocol for a randomised controlled trial of the effect of dapagliflozin, metformin and exercise on glycaemic variability, body composition and cardiovascular risk in prediabetes (the PRE-D Trial).

SGLT-2 Inhibition with Dapagliflozin Reduces the Activation of the Nlrp3/ASC Inflammasome and Attenuates the Development of Diabetic Cardiomyopathy in Mice with Type 2 Diabetes. Further Augmentation of the Effects with Saxagliptin, a DPP4 Inhibitor.
Ye Y, Bajaj M, Yang HC, Perez-Polo JR, Birnbaum Y.

Long-term treatment with the sodium glucose cotransporter 2 inhibitor, dapagliflozin, ameliorates glucose homeostasis and diabetic nephropathy in db/db mice.

Influence of Dapagliflozin on Glycemic Variations in Patients with Newly Diagnosed Type 2 Diabetes Mellitus.
Li FF1, Gao G1, Li Q1, Zhu HH1, Su XF1, Wu JD1, Ye L2, Ma JH1.
Annex

Annex 1. T02 Format

Formato_T02.-
Reporte de Reaccion


Instructivo de
Llenado T02.docx


AZ pregnancy
outcome report.pdf