Effects of SGLT2 Inhibitors on Circulating Stem and Progenitor Cells in Patients With Type 2 Diabetes

Benedetta Maria Bonora,[1] Roberta Cappellari,[1,2] Mattia Albiero,[1,2] Angelo Avogaro,[1] and Gian Paolo Fadini[1,2]

[1]Department of Medicine, University of Padova, 35128 Padova, Italy; and [2]Venetian Institute of Molecular Medicine, 35129 Padova, Italy

Context: Reduction in the levels of circulating stem cells (CSCs) and endothelial progenitor cells (EPCs) predicts development or progression of microangiopathy and macroangiopathy in patients with type 2 diabetes (T2D).

Objective: We tested whether treatment with sodium glucose cotransporter-2 (SGLT2) inhibitors affected the levels of CSCs and EPCs.

Design: A randomized trial of dapagliflozin vs placebo with open-label extension, and an open-label observational study of empagliflozin treatment.

Setting: Tertiary referral diabetes outpatient clinic.

Patients: Patients with T2D aged 18 to 75 years.

Intervention: Dapagliflozin at 10 mg vs placebo (n = 31); empagliflozin at 10 mg (n = 15).

Main Outcome Measures: We measured CSCs ($CD34^+$) and EPCs ($CD34^+KDR^+$) by flow cytometry at baseline, at 12 weeks, and after the extension period.

Results: After 12 weeks, CSCs declined nonsignificantly in the dapagliflozin group, remained stable in the placebo group, and the change from baseline was not significantly different between the two groups. EPCs declined nonsignificantly in the dapagliflozin group, increased nonsignificantly in the placebo group, and the change from baseline was significantly different between the two groups. After an open-label extension period of about 1.5 years, CSCs remained stable over time, whereas EPCs significantly increased in patients who received dapagliflozin. In all patients, irrespectively of treatment, EPCs increased significantly from baseline to the end of observation, concomitantly with improvement in HbA1c. In a cohort of 15 patients who received open-label empagliflozin for 12 weeks, CSCs declined nonsignificantly, whereas EPCs remained stable.

Conclusion: SGLT2 inhibitors do not significantly increase CSCs or EPCs. Thus, cardiovascular protection by SGLT2 inhibitors may not directly involve stem/progenitor cells. (***J Clin Endocrinol Metab* 103: 3773–3782, 2018**)

People with type 2 diabetes (T2D) experience a high rate of major adverse cardiovascular events (MACEs), which substantially contribute to morbidity and mortality (1). For this reason, glucose-lowering medications (GLMs) that, in addition to improving glucose control, also reduce the risk of MACEs are particularly attractive for the treatment of T2D (2). Sodium glucose cotransporter 2 inhibitors (SGLT2is) induce glycosuria, thereby reducing plasma glucose, blood pressure, and body weight (3). In the last few years, studies have shown that SGLT2is

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

Received 16 April 2018. Accepted 27 July 2018.
First Published Online 2 August 2018

Abbreviations: BM, bone marrow; CSC, circulating stem cell; DPP4i, dipeptidyl peptidase-4 inhibitor; EPC, endothelial progenitor cell; EPO, erythropoietin; GLM, glucose-lowering medication; HDL, high-density lipoprotein; HF, heart failure; KDR, kinase insert domain receptor; MACE, major adverse cardiovascular event; SGLT2, sodium glucose cotransporter-2; SGLT2i, sodium glucose cotransporter-2 inhibitor; T2D, type 2 diabetes.

improve cardiovascular outcomes in T2D. In the EMPA-REG Outcome trial (4), 7020 patients with T2D and additional cardiovascular risk factors or established cardiovascular disease were randomized to receive the SGLT2i empagliflozin or placebo. Empagliflozin reduced the occurrence of the 3-point MACE (composite of cardiovascular death, nonfatal myocardial infarction, and nonfatal stroke) by 14%, with a concomitant reduction in cardiovascular and all-cause mortality, and a 35% decrease in the rate of hospitalization for heart failure (HF). In the CANVAS trial program (5), 10,142 patients with T2D and high cardiovascular risk were randomized to receive the SGLT2i canagliflozin or placebo. Canagliflozin reduced the occurrence of the 3-point MACE by 14% and hospitalization for HF by 33%, although with a twofold increase in the risk of lower-limb amputations. Although the cardiovascular outcome trial on dapagliflozin is still ongoing (6), a large retrospective study reported that SGLT2is as a class, when compared with other GLMs, reduced the risk of MACEs, mortality, and HF (7–9), with consistent results among dapagliflozin, empagliflozin, and canagliflozin.

The mechanisms whereby SGLT2is exert such striking cardiovascular protective effects are largely unknown. Several, rather complicated, explanations have been hypothesized (10, 11), but each has been criticized (12) or dismantled (13). Because cardiovascular protection by SGLT2is is mostly evident for HF, the diuretic effect remains a solid explanation (14).

In the last 10 years, it has been recognized that, in addition to increasing vascular damage, diabetes compromises vascular repair (15). Circulating, bone marrow (BM)–derived stem and progenitor cells, which are provided with vasculotrophic functions (16), are reduced by ~40% in T2D (17), and this is thought to contribute to cardiovascular risk (18). This cellular population includes CD34-expressing circulating stem cells (CSCs), mostly of hematopoietic origin, and a subset coexpressing endothelial markers such as vascular endothelial growth factor receptor 2 [or kinase insert domain receptor (KDR)], which have been deemed endothelial progenitor cells (EPCs) (19). In patients with T2D, a reduction in the levels of CSCs predicted adverse cardiovascular outcomes, defined as the 3-point MACE plus hospitalization for cardiovascular causes (20). Other longitudinal studies have shown that patients with a reduction in the levels of CSCs or EPCs have a worse prognosis in terms of cardiovascular events, cardiovascular death, and all-cause mortality [meta-analyzed in Ref. (21)]. Some therapeutic strategies proved able to stimulate CSCs or EPCs in T2D (22, 23), although the clinical implications of such effect is unclear.

In this study, we tested the hypothesis that SGLT2i can increase CSCs and/or EPCs, which would represent a novel potential mechanism of cardiovascular protection by these drugs.

Materials and Methods

The study flowchart is depicted in Fig. 1.

Effects of 12 weeks of dapagliflozin vs placebo

The NCT02327039 study was approved by the Ethical Committee of the University Hospital of Padova and conducted according to national and international standards. All procedures involving human subjects were carried out in accordance with the Declaration of Helsinki. A total of 33 patients with T2D were randomly assigned to receive dapagliflozin at 10 mg (n = 17) or matching placebo (n = 16) on top of their glucose-lowering medications for 12 weeks. Two patients in the dapagliflozin group were excluded because of withdrawn consent or were lost to follow-up, leaving 31 completers. The primary endpoint was the change from baseline in cholesterol

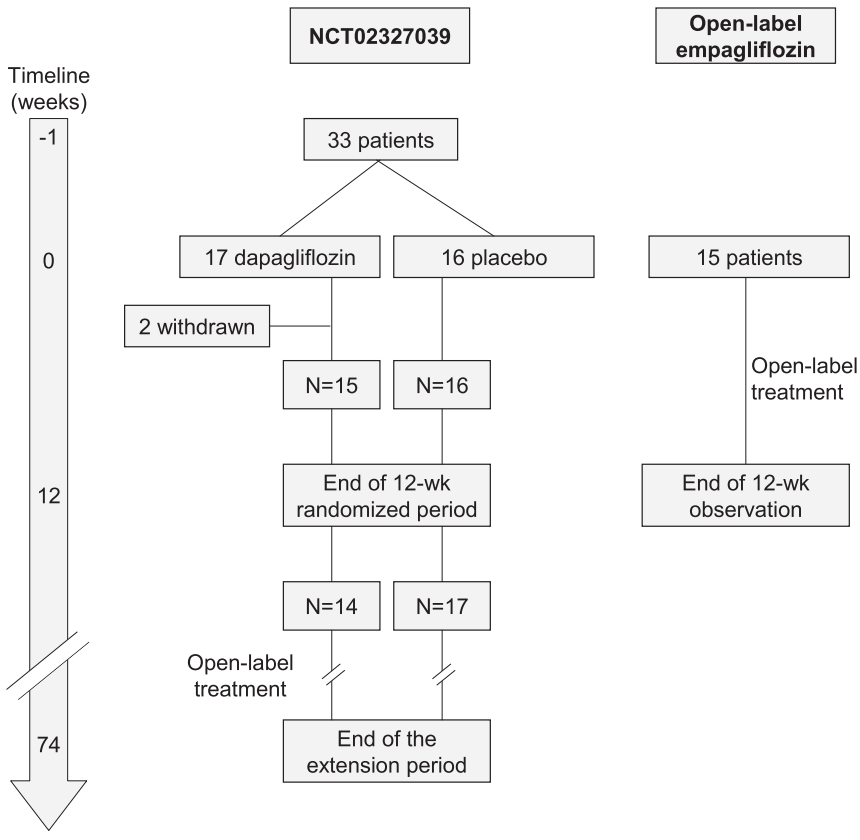

Figure 1. Study flowchart.

efflux capacity. Study details and the primary results have already been published (24). We herein report results of a secondary endpoint, that is, the change from baseline to 12 weeks in the levels of CSCs and EPCs, quantified by flow cytometry (see below).

Briefly, inclusion criteria were: females or males aged 18 to 75 years, diagnosis of T2D at least 6 months earlier, and underlying therapy with oral glucose-lowering medications and/or insulin. Exclusion criteria were: acute illness or infection; recent surgery, trauma, or cardiovascular event; recent variation of statin therapy/dose; alcoholism; very high baseline high-density lipoprotein (HDL) cholesterol levels (>90 mg/dL); previous history of recurrent urinary tract infections or genital infections; history of hypotension or episodes of volume depletion/dehydration; chronic kidney disease (estimated glomerular filtration rate <60 mL/min/1.73 mq); chronic liver disease; HF New York Heart Association classes III to IV; ongoing treatment with pioglitazone or GLP-1 receptor agonists; and pregnancy or lactation. All patients provided written informed consent.

Open-label extension

As an extension of the NCT02327039 study, after the 12-week blinded period, patients on open-label treatment with or without dapagliflozin were followed until the last available visit and CSC/EPC levels were measured again.

Effects of 12 weeks of open-label empagliflozin

To gather insight into the effects of another SGLT2i, we included an additional equal number (n = 15) of patients with T2D who were scheduled to receive empagliflozin at 10 mg in association with metformin and/or insulin, which were the combinations allowed by the Italian National Health Care System for reimbursement. Other inclusion/exclusion criteria were the same as in the placebo-controlled trial with dapagliflozin (NCT02327039). Before and 12 weeks after initiation of empagliflozin, blood samples were collected for the determination of CSCs and EPCs.

Flow cytometry

CSCs and EPCs were measured as previously described (25). Briefly, EDTA-anticoagulated blood samples collected from an antecubital vein in the fasting state were freshly analyzed within 2 hours. After red blood cell lysis, cells were stained with a fluorescein isothiocyanate–conjugated anti-human CD34 antibody (Becton Dickinson) and a PE-conjugated anti-human KDR/vascular endothelial growth factor receptor 2. A total of 5×10^5 cells were acquired on a FACSCanto II (BD Biosciences). We first gated mononuclear cells in the side light scatter area vs forward light scatter area plot and then examined the resulting population for the expression of CD34 (CSCs) and dual expression of CD34 and KDR (EPCs). The number of positive events was expressed relative to 10^6 total events. To assess reproducibility, the level of $CD34^+$ CSCs was determined in triplicate using different fluorochromes: the average coefficient of variation was 10.7%, implying good reproducibility.

Quantification of erythropoietin concentrations

An ELISA (EBMS2035, Thermo Fisher Scientific) was used for the quantitative detection of human erythropoietin (EPO) in EDTA-anticoagulated plasma samples of patients treated with dapagliflozin (randomized and extension period) or empagliflozin.

Statistical analysis

Data were expressed as mean ± SE, or as percentage where appropriate. Nonnormal continuous variables were log transformed before statistical analysis. Comparisons between two groups were analyzed using the two-tailed unpaired Student *t* test for continuous variables and the χ^2 test for categorical variables. For study endpoints, we compared baseline with follow-up data using the two-tailed paired Student *t* test and calculated the change from baseline. Then, the changes from baseline in each group were compared using the two-tailed unpaired Student *t* test. Linear correlations were analyzed using the Pearson *r* coefficient. Statistical significance was accepted at $P < 0.05$.

Results

Effects of dapagliflozin on stem/progenitor cells in 12 weeks

The baseline characteristics of patients enrolled in the NCT02327039 trial have already been described in detail (24) and are not reported here to avoid duplication. After 12 weeks, dapagliflozin reduced HbA1c by 0.9%, body weight by 3.1 kg, and systolic blood pressure by 4.7 mm Hg. No significant change was detected in lipid parameters and cholesterol efflux capacity (24).

In this study, we examined whether therapy with dapagliflozin vs placebo modified the levels of CSCs and EPCs. Compared with baseline, $CD34^+$ CSCs declined nonsignificantly during therapy with dapagliflozin (from $321.8 \pm 26.5/10^6$ to $270.8 \pm 29.3/10^6$; $P = 0.13$) and remained stable in the placebo group (from $328.3 \pm 29.4/10^6$ to $332.4 \pm 28.8/10^6$; $P = 0.89$; Fig. 2A). The change from baseline in the two groups was not significantly different ($-51.0 \pm 32.0/10^6$ in the dapagliflozin group vs $4.1 \pm 28.5/10^6$ in the placebo group; $P = 0.21$; Fig. 2B).

$CD34^+KDR^+$ EPCs declined nonsignificantly during therapy with dapagliflozin (from $13.8 \pm 2.2/10^6$ to $8.7 \pm 1.9/10^6$; $P = 0.11$) and increased nonsignificantly during therapy with placebo (from $10.5 \pm 1.9/10^6$ to $15.9 \pm 3.1/10^6$; $P = 0.19$; Fig. 2C). The change from baseline was significantly different between the two groups ($-5.1 \pm 3.0/10^6$ in the dapagliflozin group vs $5.3 \pm 3.9/10^6$ in the placebo group; $P = 0.042$; Fig. 2D).

Considering all patients irrespective of the assigned treatment (dapagliflozin or placebo), CSCs did not vary significantly from baseline to 12 weeks, but the change from baseline in CSC levels was inversely related to the change from baseline in HDL cholesterol ($r = -0.47$; $P = 0.003$) and ApoA1 concentrations ($r = -0.34$; $P = 0.045$), whereas they were directly correlated to the change from baseline in IL-6 ($r = 0.43$; $P = 0.007$).

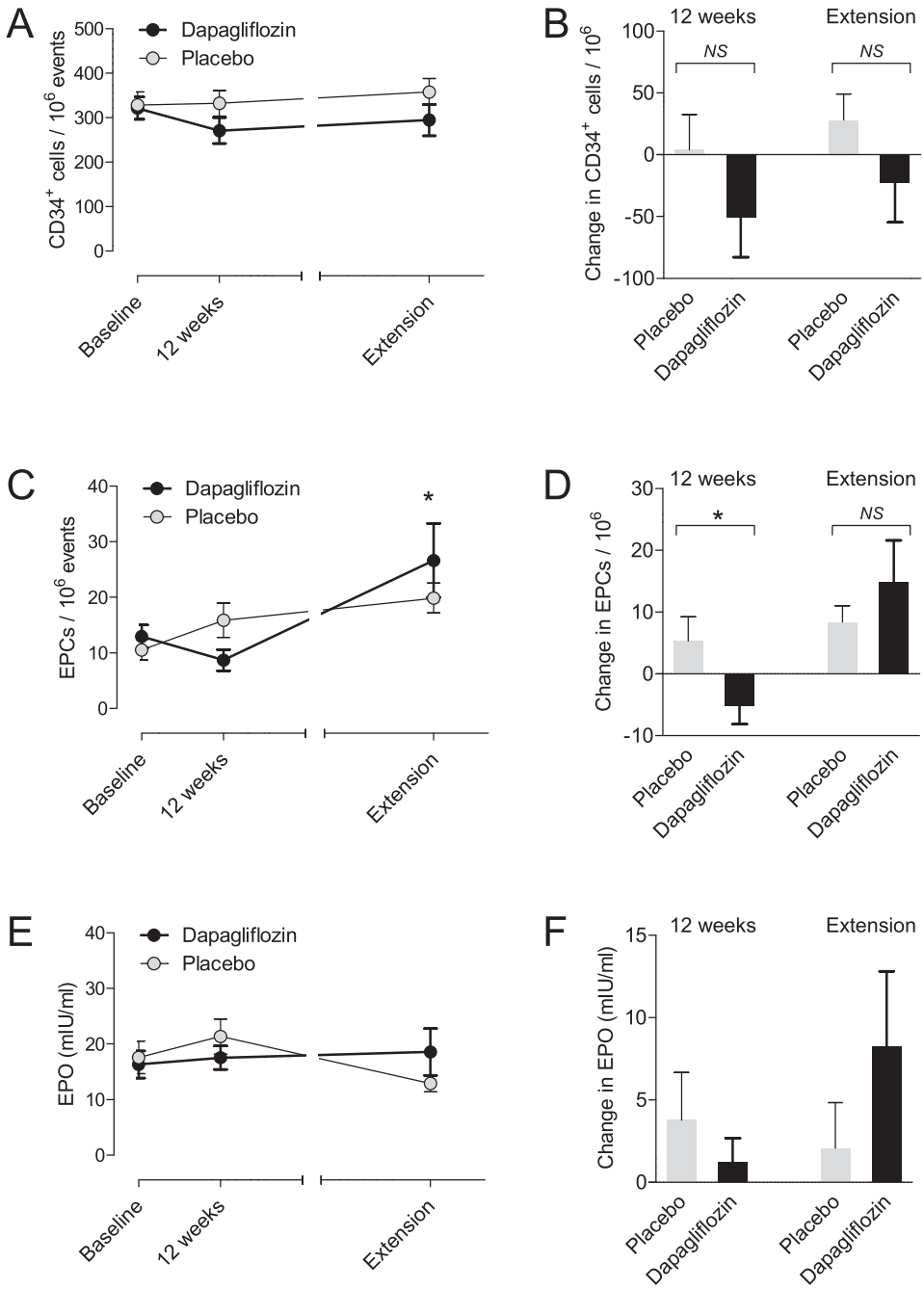

Figure 2. Effects of dapagliflozin on stem/progenitor cells. Time course of $CD34^+$ CSC levels (A), $CD34^+KDR^+$ EPC levels (C), and EPO concentrations (E) during treatment with dapagliflozin or placebo in the NCT02327039 trial and during the extension period. EPCs at the end of the extension period were significantly higher (*$P < 0.05$) vs levels at baseline and vs levels at 12 wk. The change from baseline in CSC levels (B), EPC levels (D), and EPO concentrations (F) is reported for the 12 wk of randomized treatment and for the extension period. *$P < 0.05$ for the indicated comparison. NS, not significant.

Overall, EPC levels did not change in the entire study cohort and no correlation was detected between the change from baseline in EPC levels and the change in biochemical parameters, cytokines, or adipokine concentrations.

Effects of longer treatment with dapagliflozin on stem/progenitor cells

Because we detected no clear effect of dapagliflozin on CSCs and EPCs during a short time course, after conclusion of the 12-week blinded treatment, we prolonged the observation while patients were (n = 14) or were not (n = 17) on open-label dapagliflozin and repeated the quantification of stem/progenitor cells. During this extension period (median, 17.1 months or 74 weeks; interquartile range, 14.8 to 21.1 months), HbA1c remained stable in patients who were on dapagliflozin (from 7.3% ± 0.2% to 7.4% ± 0.2%; $P = 0.68$) and declined nonsignificantly in patients who were not on

dapagliflozon (from 8.6% ± 0.4% to 8.0% ± 0.6%; P = 0.122). CSCs increased nonsignificantly both in patients who were and in those who were not on dapagliflozin. CSC levels at the end of observation were not significantly different between the two groups and vs baseline in both groups (Fig. 2A and 2B).

During the extension period, EPCs increased significantly in patients who received dapagliflozin and nonsignificantly in patients who did not receive dapagliflozin. EPC levels at the end of observation were not significantly different between the two groups and vs baseline in both groups (Fig. 2C and 2D).

When the time course of CSCs and EPCs was examined over the blinded plus extension period for the entire cohort, irrespective of whether patients did or did not receive dapagliflozin, EPC levels increased significantly at the end of observation compared with baseline and compared with the end of the blinded period (Fig. 3).

Effects of empagliflozin on stem/progenitor cells in 12 weeks

Because we did not detect significant effects of dapagliflozin on CPCs and EPCs in the 12-week placebo-controlled trial and during open-label extension, we evaluated whether a different SGLT2i would exert different effects. Thus, we quantified CSCs and EPCs at baseline and at 12 weeks in patients who were scheduled to receive empagliflozin at 10 mg in association with metformin and/or insulin. Table 1 summarizes clinical characteristics of patients who received dapagliflozin in the NCT02327039 trial and open-label empagliflozin. Except for some expected differences in concomitant medications, the two groups of patients were very well balanced, implying that the results could be compared and pooled. Empagliflozin reduced HbA1c from 8.0% to 7.2% (−0.8%) and body weight from 84.3 to 80.6 kg (−3.6 kg). Compared with baseline, CSCs declined nonsignificantly (from 281.9 ± 35.2/10^6 to 222.8 ± 31.1/10^6; P = 0.22; Fig. 4A) and EPCs remained stable (from 11.0 ± 1.8/10^6 to 11.5 ± 1.5/10^6, P = 0.82: Fig. 4B).

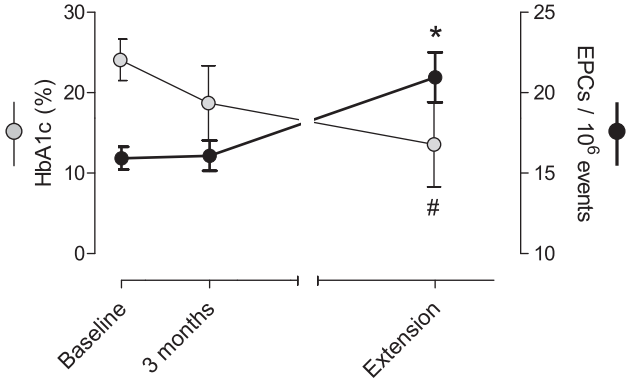

Figure 3. Time course of EPC levels in relationship to HbA1c. The entire study cohort of n = 31 patients of the trial, NCT02327039, was examined irrespective of the assigned treatment. At the end of the extension period, EPCs were increased significantly vs baseline (*$P < 0.05$) and HbA1c was reduced significantly vs baseline (#$P < 0.05$).

Because a trend toward reduction of CSCs from baseline to 12 weeks was noted during treatment with both dapagliflozin and empagliflozin, we performed a pooled analysis: CSC levels in patients treated for 12 weeks with dapagliflozin or empagliflozin declined from 304.0 ± 20.9/10^6 to 249.3 ± 21.5/10^6, a difference that was still not statistically significant (P = 0.052; Fig. 4D). EPCs declined nonsignificantly from 12.1 ± 1.4/10^6 to 10.0 ± 1.3/10^6; P = 0.18 (Fig. 4E).

Concentrations of EPO did not change significantly during the 12-week period treatment with dapagliflozin nor during the extension period (Fig. 2E and 2F). However, in the pooled analysis of patients treated with dapagliflozin or empagliflozin for 12 weeks, we found a significant increase in EPO concentrations (Fig. 4C and 4F).

Discussion

Reduction in the levels of CSCs and EPCs is a consistent observation in people with T2D, and there is a consensus that this defect contributes to the development of chronic complications (26). In particular, low CSCs have been shown to predict onset or worsening of microangiopathy and future adverse cardiovascular outcomes (20, 27). Two cardiovascular outcome trials and one cohort study demonstrate that SGLT2is as a class reduce the risk of cardiovascular events in patients with T2D and high cardiovascular risk (4, 5, 9). Despite several theoretical and experimental attempts to explain such striking cardiovascular protection exerted by SGLT2is, we still have a poor understanding of the potential mechanisms at work.

Therefore, it was reasonable to test whether SGLT2is exerted beneficial effects on stem/progenitor cells, which are considered both biomarkers and actors in the pathogenesis of chronic diabetic complications. Our data reasonably rule out that SGLT2is can directly increase CSCs or EPCs. We first quantified CSCs and EPCs during a 12-week randomized placebo-controlled trial of dapagliflozin treatment. After having shown no significant effect of dapagliflozin vs placebo on CSCs and EPCs during this short time course, we took two additional strategies. First, we evaluated the effectiveness of an alternative SGLT2i, empagliflozin, for which more robust data on cardiovascular protection were available. Again, a 12-week treatment with empagliflozin did not

Table 1. Clinical Characteristics of Patients Who Received Dapagliflozin or Empagliflozin

	Any SGLT2i	Dapagliflozin	Empagliflozin	*P*
Number	30	15	15	
Anthropometrics				
Age, y	66.0 ± 1.3	66.3 ± 1.5	65.7 ± 2.2	0.824
Male sex, %	73.3	68.8	78.6	0.560
Body weight, kg	84.2 ± 2.9	84.2 ± 4.0	84.3 ± 4.4	0.987
Height, cm	169.4 ± 1.8	171.9 ± 2.1	166.5 ± 2.8	0.127
BMI, kg/m^2	31.1 ± 2.1	28.4 ± 1.1	30.1 ± 1.1	0.290
Disease duration, y	14.5 ± 1.3	14.2 ± 1.9	14.8 ± 1.9	0.826
HbA1c, %	8.1 ± 0.1	8.2 ± 0.1	8.0 ± 0.2	0.602
Concomitant risk factors				
Total cholesterol, mg/dL	160.0 ± 6.2	168.1 ± 7.8	150.6 ± 9.7	0.166
HDL cholesterol, mg/dL	47.1 ± 2.3	49.7 ± 3.1	44.1 ± 3.3	0.228
LDL cholesterol, mg/dL	87.2 ± 5.4	95.6 ± 6.9	77.5 ± 7.9	0.093
Triglycerides, mg/dL	138.4 ± 19.0	133.4 ± 26.0	144.2 ± 28.9	0.782
Hypertension, %	90.0	87.5	92.9	0.640
Microangiopathy, %	30.0	25.0	50.0	0.167
Albumin/creatinine ratio, mg/g	22.5 ± 6.8	22.9 ± 10.9	22.1 ± 8.2	0.952
Serum creatinine, mg/dL	0.8 ± 0.0	0.8 ± 0.0	0.9 ± 0.0	0.507
eGFR, ml/min/1.73 m^2	90.0 ± 3.5	89.3 ± 4.1	90.7 ± 6.0	0.845
Retinopathy, %	23.3	18.8	28.6	0.542
Nephropathy, %	6.7	12.5	21.4	0.183
Neuropathy, %	10.0	0.0	21.4	0.053
Macroangiopathy, %	70.0	62.5	78.6	0.355
Coronary artery disease, %	30.0	12.5	50.0	0.025
Peripheral arterial disease, %	10.0	0.0	21.4	0.053
Cerebrovascular disease, %	63.3	62.5	64.3	0.923
Concomitant GLM				
Metformin, %	90.0	87.5	92.9	0.640
Sulphonylurea, %	10.0	18.8	0.0	0.093
DPP4is, %	13.3	25.0	0.0	0.046
Insulin, %	53.3	43.8	64.3	0.276
Other medications				
ACEi /ARBs, %	83.3	75.0	92.9	0.203
Other drugs for blood pressure, %	53.3	50.0	57.1	0.708
Statin, %	86.7	81.3	92.9	0.368
APA, %	66.7	43.8	92.9	0.003

P values indicate the comparison between patients who received dapagliflozin in the NCT02327039 trial and those who received open-label empagliflozin. The comparison between patients who received dapagliflozin vs placebo in the NCT02327039 trial has been published previously (24). None of the comparison with nominal $P < 0.05$ remained significant after adjustment for multiple comparisons using the Bonferroni test.

Abbreviations: APA, antiplatelet agent; BMI, body mass index; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein.

significantly change CSC or EPC levels. Second, we prolonged the observation of patients treated with dapagliflozin for almost 1.5 years. We found that, with much longer observation and a stable HbA1c decline, EPCs were significantly increased compared with baseline and compared with the end of the initial 12-week period. However, a more modest increase was also noted in patients who did not receive dapagliflozin and yet experienced a modest decline in HbA1c. Therefore, such an increase in EPC levels is unlikely to be attributable to SGLT2is *per se*, but more likely mediated by the improved glucose control. Indeed, we previously observed a quite similar trend in patients with poorly controlled T2D undergoing optimization of glucose control by add-on basal insulin: despite that HbA1c declined within the first 3 months, EPCs did not change, and at least 6 months of good glycemic control was needed to raise EPC levels (28). This concept is further supported, in the current study, by the observation that, irrespective of therapy, EPCs increased as HbA1c declined during the extension period, supporting that an improvement in glucose control takes time to modulate EPC levels. Nonetheless, we explored an alternative explanation. In a reanalysis of the EMPA-REG Outcome trial (29), change from baseline in hematocrit mediated >50% of the effect of empagliflozin vs placebo on the risk of cardiovascular death. Because EPO regulates both erythropoiesis and stem/progenitor cells (30), we quantified EPO levels. Although EPO levels increased during a 12-week treatment with SGLT2i (pooled analysis), no increase occurred during the extension period, thereby ruling out a direct relationship between EPO and EPC increase.

The scenario presented in the present study seems opposite to that observed with dipeptidyl peptidase-4

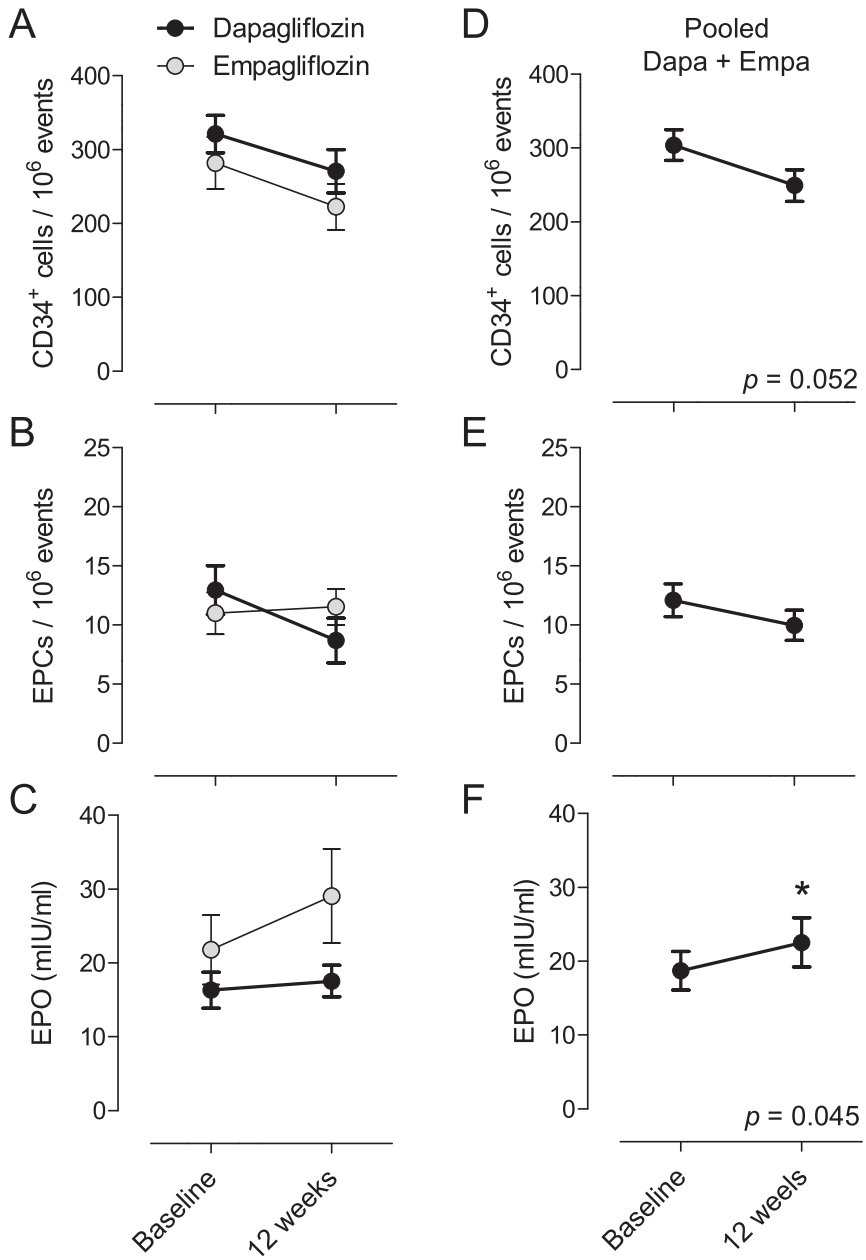

Figure 4. Effects of 12 wk of dapagliflozin/empagliflozin separately and pooled. The change in $CD34^+$ CSCs (A), $CD34^+KDR^+$ EPCs (B), and EPO concentrations (C) in patients receiving empagliflozin for 12 wk is reported and compared with the effects of dapagliflozin in the NCT02327039 trial. The pooled effects of dapagliflozin or empagliflozin treatment of 12 wk on CSCs (C), EPCs (D) and EPO (F) are also shown. $^*P < 0.05$.

inhibitors (DPP4is) (22). By protecting SDF-1α from enzymatic degradation, DPP4is raise active SDF-1α concentrations, which in turn recruits EPCs into the circulation (31). This effect has been consistently demonstrated for sitagliptin, linagliptin, and vildagliptin, occurs in just a few days, persists over time, and is independent from glucose control (32–34).

Intriguingly, although reductions in CSCs and EPCs predict future cardiovascular events (20, 21), drugs that stimulate these cells (DPP4is) have failed to show cardiovascular benefits in large placebo-controlled trials (35–37). Alternatively, drugs that have been consistently shown to convey cardiovascular protection (SGLT2is) (4, 5) do not appear to directly stimulate CSCs and EPCs. Overall, this observation may question the hypothesis that reduction in stem/progenitor cells is a modifiable risk factor or, in other terms, that increasing CSCs/EPCs reduces the patient's cardiovascular risk. In recent years, it has been clarified that pauperization of CSCs in diabetes results from structural and functional alterations of the BM, including microangiopathy, neuropathy, and inflammation (26, 38). Therefore, only therapies targeting such BM alterations may result in a stable reversal of the CSC defect and eventually modify the patient's outcomes. This may explain why glucose control takes time to raise EPCs.

Despite these speculations, note that the ability of SGLT2is to improve cardiovascular outcomes in large trials was mostly attributable to a strong protection against HF (39, 40). Although low CSC counts have been shown to predict mortality in patients with HF (41), no study has clearly demonstrated whether stem/progenitor cell levels also predict onset of HF. Unexpectedly, in patients who received either dapagliflozin or empagliflozin, CSCs tended to decline from baseline to week 12, and the pooled analysis of the two patient groups yielded a nearly significant result. Based on available longitudinal studies (20, 21), this finding would paradoxically imply an increase in the rate of MACEs and it is also difficult to interpret in relationship to HF risk, because a biphasic association has been described between HF and CSC levels, with CSC increase in the early stages and CSC decline in the later stages of HF (42, 43). Finally, a clear signal has emerged that the SGLT2i canagliflozin may increase the risk of amputation (5, 44, 45). Although we did not evaluated canagliflozin in the current study, it is possible that reduction in CSCs during therapy with SGLT2i contributed to an eventual worsening of diabetic foot (46, 47), thereby leading to amputation.

Results of our study need to be interpreted in view of some limitations. Although sample size was relatively small, we have previously demonstrated the stimulatory effects of DPP4i on EPCs with similar numbers of patients (34). Based on the pooled analysis of patients receiving dapagliflozin or empagliflozin for 12 weeks, and assuming that the observed CSC reduction of about 50 cells/10^6 persisted with more observations, we calculate that a total of 250 patients would be needed to reach statistical significance with 80% power. In other terms, although the observed CSC reduction of 50 cells/10^6 was nearly significant, power calculated *a posteriori* was ~15%. Thus, from a statistical viewpoint, the apparent reduction in CSCs during therapy with SGLT2is is likely to be a chance effect. Rather, during the 12-week period, CSCs appeared to change irrespectively of therapy and in relationship to HDL and IL-6. This is consistent with the notion that HDL particles regulate stem cell kinetics and inflammatory myelopoiesis (48, 49). Furthermore, we recognize that empagliflozin treatment was not randomized and that associated therapies were different from those in the dapagliflozin trial. Despite the use of DPP4i, which stimulates EPCs (22), was more common in association with dapagliflozin than with empagliflozin (Table 1), and this unlikely affected results, as neither SGLT2i increased EPCs. Similarly, although a history of cardiovascular disease can affect EPCs (50), the different distribution of macroangiopathies in the two groups can hardly explain why SGLT2is did not increase EPCs.

In conclusion, we found no evidence that SGLT2is can directly improve stem/progenitor cell levels in T2D. Thus, cardiovascular protection elicited by SGLT2is should be mediated by other mechanisms. Comparatively, based on the highly consistent observation that DPP4is increase EPCs but do not improve cardiovascular outcomes, we speculate that simply increasing progenitor cells in the blood without counteracting adverse BM remodeling may be insufficient to improve clinically relevant outcome.

Acknowledgments

Financial Support: This work was supported in part by a grant from AstraZeneca to A.A. The sponsor had no role in study design and conduction, manuscript preparation, and decision to publish. The study was also supported by grants from the University of Padova (BIRD) and the Italian Ministry of Education (PRIN2015ZTT5KB) to G.P.F.

Correspondence and Reprint Requests: Gian Paolo Fadini, MD PhD, Department of Medicine, University of Padova, Via Giustiniani 2, 35128 Padova, Italy. E-mail: gianpaolo.fadini@unipd.it.

Disclosure Summary: G.P.F. received grant support, lecture fees, or advisory board fees from AstraZeneca, Boehringer Ingelheim, Eli Lilly, Novo Nordisk, Sanofi, Genzyme, Abbott, Novartis, and Merck Sharp & Dohme. A.A. received research grants, lecture fees, or advisory board fees from Merck Sharp & Dome, AstraZeneca, Novartis, Boeringher Ingelheim, Sanofi, Mediolanum, Janssen, Novo Nordisk, Eli Lilly, Servier, and Takeda. The remaining authors have nothing to disclose.

References

1. Rao Kondapally Seshasai S, Kaptoge S, Thompson A, Di Angelantonio E, Gao P, Sarwar N, Whincup PH, Mukamal KJ, Gillum RF, Holme I, Njølstad I, Fletcher A, Nilsson P, Lewington S, Collins R, Gudnason V, Thompson SG, Sattar N, Selvin E, Hu FB, Danesh J; Emerging Risk Factors Collaboration. Diabetes mellitus, fasting glucose, and risk of cause-specific death. *N Engl J Med*. 2011;**364**(9):829–841.
2. Fox CS, Golden SH, Anderson C, Bray GA, Burke LE, de Boer IH, Deedwania P, Eckel RH, Ershow AG, Fradkin J, Inzucchi SE, Kosiborod M, Nelson RG, Patel MJ, Pignone M, Quinn L, Schauer PR, Selvin E, Vafiadis DK; American Heart Association Diabetes Committee of the Council on Lifestyle and Cardiometabolic Health; Council on Clinical Cardiology, Council on Cardiovascular and Stroke Nursing, Council on Cardiovascular Surgery and Anesthesia, Council on Quality of Care and Outcomes Research; American Diabetes Association. Update on prevention of cardiovascular disease in adults with type 2 diabetes mellitus in light of recent evidence: a scientific statement from the American Heart Association and the American Diabetes Association. *Diabetes Care*. 2015;**38**(9):1777–1803.
3. Li J, Gong Y, Li C, Lu Y, Liu Y, Shao Y. Long-term efficacy and safety of sodium-glucose cotransporter-2 inhibitors as add-on to metformin treatment in the management of type 2 diabetes mellitus: A meta-analysis. *Medicine (Baltimore)*. 2017;**96**(27):e7201.
4. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, Mattheus M, Devins T, Johansen OE, Woerle HJ, Broedl UC, Inzucchi SE; EMPA-REG OUTCOME Investigators. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. 2015;**373**(22):2117–2128.
5. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, Shaw W, Law G, Desai M, Matthews DR; CANVAS Program Collaborative Group. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med*. 2017;**377**(7):644–657.
6. Raz I, Mosenzon O, Bonaca MP, Cahn A, Kato ET, Silverman MG, Bhatt DL, Leiter LA, McGuire DK, Wilding JPH, Gause-Nilsson IAM, Langkilde AM, Johansson PA, Sabatine MS, Wiviott SD. DECLARE-TIMI 58: Participants' baseline characteristics. *Diabetes Obes Metab*. 2018;**20**(5):1102–1110.
7. Birkeland KI, Jørgensen ME, Carstensen B, Persson F, Gulseth HL, Thuresson M, Fenici P, Nathanson D, Nyström T, Eriksson JW, Bodegård J, Norhammar A. Cardiovascular mortality and morbidity in patients with type 2 diabetes following initiation of sodium-glucose co-transporter-2 inhibitors versus other glucose-lowering drugs (CVD external-REAL Nordic): a multinational observational analysis. *Lancet Diabetes Endocrinol*. 2017;**5**(9):709–717.
8. Persson F, Nyström T, Jørgensen ME, Carstensen B, Gulseth HL, Thuresson M, Fenici P, Nathanson D, Eriksson JW, Norhammar A, Bodegard J, Birkeland KI. Dapagliflozin is associated with lower risk of cardiovascular events and all-cause mortality in people with type 2 diabetes (CVD-REAL Nordic) when compared with dipeptidyl peptidase-4 inhibitor therapy: a multinational observational study. *Diabetes Obes Metab*. 2018;**20**(2):344–351.
9. Kosiborod M, Cavender MA, Fu AZ, Wilding JP, Khunti K, Holl RW, Norhammar A, Birkeland KI, Jørgensen ME, Thuresson M, Arya N, Bodegård J, Hammar N, Fenici P; CVD-REAL Investigators and Study Group. Lower risk of heart failure and death

in patients initiated on sodium-glucose cotransporter-2 inhibitors versus other glucose-lowering drugs: the CVD-REAL study (comparative effectiveness of cardiovascular outcomes in new users of sodium-glucose cotransporter-2 inhibitors). *Circulation*. 2017;**136**(3):249–259.
10. Packer M. Do sodium-glucose co-transporter-2 inhibitors prevent heart failure with a preserved ejection fraction by counterbalancing the effects of leptin? A novel hypothesis. *Diabetes Obes Metab*. 2018;**20**(6):1361–1366.
11. Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: a "thrifty substrate" hypothesis. *Diabetes Care*. 2016;**39**(7):1108–1114.
12. Lopaschuk GD, Verma S. Empagliflozin's fuel hypothesis: not so soon. *Cell Metab*. 2016;**24**(2):200–202.
13. Avogaro A, Fadini GP. Counterpoint to the hypothesis that SGLT2 inhibitors protect the heart by antagonizing leptin. *Diabetes Obes Metab*. 2018;**20**(6):1367–1368.
14. Hallow KM, Helmlinger G, Greasley PJ, McMurray JJV, Boulton DW. Why do SGLT2 inhibitors reduce heart failure hospitalization? A differential volume regulation hypothesis. *Diabetes Obes Metab*. 2018;**20**(3):479–487.
15. Schaper NC, Havekes B. Diabetes: impaired damage control. *Diabetologia*. 2012;**55**(1):18–20.
16. Pozzoli O, Vella P, Iaffaldano G, Parente V, Devanna P, Lacovich M, Lamia CL, Fascio U, Longoni D, Cotelli F, Capogrossi MC, Pesce M. Endothelial fate and angiogenic properties of human $CD34^+$ progenitor cells in zebrafish. *Arterioscler Thromb Vasc Biol*. 2011;**31**(7):1589–1597.
17. Fadini GP, Boscaro E, de Kreutzenberg S, Agostini C, Seeger F, Dimmeler S, Zeiher A, Tiengo A, Avogaro A. Time course and mechanisms of circulating progenitor cell reduction in the natural history of type 2 diabetes. *Diabetes Care*. 2010;**33**(5):1097–1102.
18. Fadini GP, Ferraro F, Quaini F, Asahara T, Madeddu P. Concise review: diabetes, the bone marrow niche, and impaired vascular regeneration. *Stem Cells Transl Med*. 2014;**3**(8):949–957.
19. Fadini GP, Losordo D, Dimmeler S. Critical reevaluation of endothelial progenitor cell phenotypes for therapeutic and diagnostic use. *Circ Res*. 2012;**110**(4):624–637.
20. Fadini GP, Rigato M, Cappellari R, Bonora BM, Avogaro A. Long-term prediction of cardiovascular outcomes by circulating $CD34^+$ and $CD34^+CD133^+$ stem cells in patients with type 2 diabetes. *Diabetes Care*. 2017;**40**(1):125–131.
21. Rigato M, Avogaro A, Fadini GP. Levels of circulating progenitor cells, cardiovascular outcomes and death: a meta-analysis of prospective observational studies. *Circ Res*. 2016;**118**(12):1930–1939.
22. Fadini GP, Avogaro A. Dipeptidyl peptidase-4 inhibition and vascular repair by mobilization of endogenous stem cells in diabetes and beyond. *Atherosclerosis*. 2013;**229**(1):23–29.
23. Fadini GP, Avogaro A. Potential manipulation of endothelial progenitor cells in diabetes and its complications. *Diabetes Obes Metab*. 2010;**12**(7):570–583.
24. Fadini GP, Bonora BM, Zatti G, Vitturi N, Iori E, Marescotti MC, Albiero M, Avogaro A. Effects of the SGLT2 inhibitor dapagliflozin on HDL cholesterol, particle size, and cholesterol efflux capacity in patients with type 2 diabetes: a randomized placebo-controlled trial. *Cardiovasc Diabetol*. 2017;**16**(1):42.
25. Fadini GP, Boscari F, Cappellari R, Galasso S, Rigato M, Bonora BM, D'Anna M, Bruttomesso D, Avogaro A. Effects of hypoglycemia on circulating stem and progenitor cells in diabetic patients. *J Clin Endocrinol Metab*. 2018;**103**(3):1048–1055.
26. Fadini GP, Ciciliot S, Albiero M. Concise review: perspectives and clinical implications of bone marrow and circulating stem cell defects in diabetes. *Stem Cells*. 2017;**35**(1):106–116.
27. Rigato M, Bittante C, Albiero M, Avogaro A, Fadini GP. Circulating progenitor cell count predicts microvascular outcomes in type 2 diabetic patients. *J Clin Endocrinol Metab*. 2015;**100**(7):2666–2672.
28. Fadini GP, de Kreutzenberg SV, Mariano V, Boscaro E, Bertolini F, Mancuso P, Quarna J, Marescotti M, Agostini C, Tiengo A, Avogaro A. Optimized glycaemic control achieved with add-on basal insulin therapy improves indexes of endothelial damage and regeneration in type 2 diabetic patients with macroangiopathy: a randomized crossover trial comparing detemir versus glargine. *Diabetes Obes Metab*. 2011;**13**(8):718–725.
29. Inzucchi SE, Zinman B, Fitchett D, Wanner C, Ferrannini E, Schumacher M, Schmoor C, Ohneberg K, Johansen OE, George JT, Hantel S, Bluhmki E, Lachin JM. How does empagliflozin reduce cardiovascular mortality? Insights from a mediation analysis of the EMPA-REG OUTCOME trial. *Diabetes Care*. 2018;**41**(2):356–363.
30. Bahlmann FH, De Groot K, Spandau JM, Landry AL, Hertel B, Duckert T, Boehm SM, Menne J, Haller H, Fliser D. Erythropoietin regulates endothelial progenitor cells. *Blood*. 2004;**103**(3):921–926.
31. Fadini GP, Avogaro A. How to interpret the role of SDF-1α on diabetic complications during therapy with DPP-4 inhibitors. *Cardiovasc Diabetol*. 2018;**17**(1):22.
32. Dei Cas A, Spigoni V, Cito M, Aldigeri R, Ridolfi V, Marchesi E, Marina M, Derlindati E, Aloe R, Bonadonna RC, Zavaroni I. Vildagliptin, but not glibenclamide, increases circulating endothelial progenitor cell number: a 12-month randomized controlled trial in patients with type 2 diabetes. *Cardiovasc Diabetol*. 2017;**16**(1):27.
33. Fadini GP, Bonora BM, Cappellari R, Menegazzo L, Vedovato M, Iori E, Marescotti MC, Albiero M, Avogaro A. Acute effects of linagliptin on progenitor cells, monocyte phenotypes, and soluble mediators in type 2 diabetes. *J Clin Endocrinol Metab*. 2016;**101**(2):748–756.
34. Fadini GP, Boscaro E, Albiero M, Menegazzo L, Frison V, de Kreutzenberg S, Agostini C, Tiengo A, Avogaro A. The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: possible role of stromal-derived factor-1α. *Diabetes Care*. 2010;**33**(7):1607–1609.
35. Green JB, Bethel MA, Armstrong PW, Buse JB, Engel SS, Garg J, Josse R, Kaufman KD, Koglin J, Korn S, Lachin JM, McGuire DK, Pencina MJ, Standl E, Stein PP, Suryawanshi S, Van de Werf F, Peterson ED, Holman RR; TECOS Study Group. Effect of sitagliptin on cardiovascular outcomes in type 2 diabetes. *N Engl J Med*. 2015;**373**(3):232–242.
36. White WB, Cannon CP, Heller SR, Nissen SE, Bergenstal RM, Bakris GL, Perez AT, Fleck PR, Mehta CR, Kupfer S, Wilson C, Cushman WC, Zannad F; EXAMINE Investigators. Alogliptin after acute coronary syndrome in patients with type 2 diabetes. *N Engl J Med*. 2013;**369**(14):1327–1335.
37. Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, Ohman P, Frederich R, Wiviott SD, Hoffman EB, Cavender MA, Udell JA, Desai NR, Mosenzon O, McGuire DK, Ray KK, Leiter LA, Raz I; SAVOR-TIMI 53 Steering Committee and Investigators. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med*. 2013;**369**(14):1317–1326.
38. Fadini GP, DiPersio JF. Diabetes mellitus as a poor mobilizer condition. *Blood Rev*. 2018;**32**(3):184–191.
39. Radholm K, Figtree G, Perkovic V, Solomon SD, Mahaffey KW, de Zeeuw D, Fulcher G, Barrett TD, Shaw W, Desai M, Matthews DR, Neal B. Canagliflozin and heart failure in type 2 diabetes mellitus: results from the CANVAS Program (Canagliflozin Cardiovascular Assessment Study). *Circulation*. 2018;**138**(5):458–468.
40. Fitchett D, Butler J, van de Borne P, Zinman B, Lachin JM, Wanner C, Woerle HJ, Hantel S, George JT, Johansen OE, Inzucchi SE; EMPA-REG OUTCOME® trial investigators. Effects of empagliflozin on risk for cardiovascular death and heart failure hospitalization across the spectrum of heart failure risk in the

EMPA-REG OUTCOME® trial. *Eur Heart J*. 2018;**39**(5):363–370.
41. Samman Tahhan A, Hammadah M, Sandesara PB, Hayek SS, Kalogeropoulos AP, Alkhoder A, Mohamed Kelli H, Topel M, Ghasemzadeh N, Chivukula K, Ko YA, Aida H, Hesaroieh I, Mahar E, Kim JH, Wilson P, Shaw L, Vaccarino V, Waller EK, Quyyumi AA. Progenitor cells and clinical outcomes in patients with heart failure. *Circ Heart Fail*. 2017;**10**(8):e004106.
42. Fortini C, Toffoletto B, Fucili A, Puppato E, Olivares A, Beltrami AP, Fiorelli V, Bergamin N, Cesselli D, Morelli C, Francolini G, Ferrari R, Beltrami CA. Circulating stem cell vary with NYHA stage in heart failure patients. *J Cell Mol Med*. 2011;**15**(8):1726–1736.
43. Nonaka-Sarukawa M, Yamamoto K, Aoki H, Nishimura Y, Tomizawa H, Ichida M, Eizawa T, Muroi K, Ikeda U, Shimada K. Circulating endothelial progenitor cells in congestive heart failure. *Int J Cardiol*. 2007;**119**(3):344–348.
44. Udell JA, Yuan Z, Rush T, Sicignano NM, Galitz M, Rosenthal N. Cardiovascular outcomes and risks after initiation of a sodium glucose cotransporter 2 inhibitor: results from the EASEL population-based cohort study (evidence for cardiovascular outcomes with sodium glucose cotransporter 2 inhibitors in the real world). *Circulation*. 2018;**137**(14):1450–1459.
45. Fadini GP, Avogaro A. SGLT2 inhibitors and amputations in the US FDA adverse event reporting system. *Lancet Diabetes Endocrinol*. 2017;**5**(9):680–681.
46. Thom SR, Hampton M, Troiano MA, Mirza Z, Malay DS, Shannon S, Jennato NB, Donohue CM, Hoffstad O, Woltereck D, Yang M, Yu K, Bhopale VM, Kovtun S, Margolis DJ. Measurements of CD34+/CD45-dim stem cells predict healing of diabetic neuropathic wounds. *Diabetes*. 2016;**65**(2):486–497.
47. Sambataro M, Seganfreddo E, Canal F, Furlan A, Del Pup L, Niero M, Paccagnella A, Gherlinzoni F, Dei Tos AP. Prognostic significance of circulating and endothelial progenitor cell markers in type 2 diabetic foot. *Int J Vasc Med*. 2014;**2014**:589412.
48. Tall AR, Yvan-Charvet L, Westerterp M, Murphy AJ. Cholesterol efflux: a novel regulator of myelopoiesis and atherogenesis. *Arterioscler Thromb Vasc Biol*. 2012;**32**(11):2547–2552.
49. Westerterp M, Gourion-Arsiquaud S, Murphy AJ, Shih A, Cremers S, Levine RL, Tall AR, Yvan-Charvet L. Regulation of hematopoietic stem and progenitor cell mobilization by cholesterol efflux pathways. *Cell Stem Cell*. 2012;**11**(2):195–206.
50. Fadini GP, Sartore S, Albiero M, Baesso I, Murphy E, Menegolo M, Grego F, Vigili de Kreutzenberg S, Tiengo A, Agostini C, Avogaro A. Number and function of endothelial progenitor cells as a marker of severity for diabetic vasculopathy. *Arterioscler Thromb Vasc Biol*. 2006;**26**(9):2140–2146.