Practical Strategies for Improving Outcomes in T2DM: the potential role of pioglitazone and DPP4 inhibitors

Stefano Del Prato[a*1] and Robert Chilton[b*1]

[a]Section of Metabolic Diseases and Diabetes, Department of Clinical and Experimental Medicine, University of Pisa, Italy.

[b]Division of Cardiology, University of Texas Health Science Center at San Antonio and South Texas Veterans Health Care System, San Antonio, TX.

[1]The authors equally contributed to this work.

[*]Corresponding authors:

Stefano Del Prato

Department of Clinical and Experimental Medicine,

University of Pisa, Italy

Tel: (050) 995103

Fax: (050) 541521

Email: stefano.delprato@med.unipi.it

Robert Chilton

University of Texas Health Science Center at San Antonio and South Texas Veterans Health Care System

San Antonio, TX

Tel: (210) 2571400

Email: chilton@uthscsa.edu

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/dom.13169

Running title: Pioglitazone and DPP4 inhibitor combination for type 2 diabetes

Abstract

T2DM is a complex disease recognizing multiple pathogenic defects responsible for the development and progression of hyperglycemia. Each of these factors can now be tackled in a more targeted manner thanks to glucose-lowering drugs made available in the past two to three decades. Recognition of the multiplicity of the mechanisms underlying hyperglycemia calls for treatments addressing more than one these mechanisms with more emphasis placed on the earlier use of combination therapies. Although chronic hyperglycemia contributes to and amplifies cardiovascular risk, several trials have failed to show a marked effect from intensive glycemic control. During the past ten years, the effect of specific glucose-lowering agents on cardiovascular risk has been explored with dedicated trials. Overall, the cardiovascular safety of the new glucose-lowering agents has been proven with some of the trials summarized in this review, showing significant reduction of cardiovascular risk. Against this background, pioglitazone, in addition to exerting a sustained glucose-lowering effect, also has ancillary metabolic actions of potential interest in addressing the cardiovascular risk of T2DM, such as preservation of beta-cell mass and function. As such it seems a logical agent to combine with other oral anti-hyperglycemic agents, including dipeptidyl peptidase-4 inhibitors (DPP4i). DPP4i, which may also have a potential to preserve beta-cell function, is available as a fixed-dose combination with pioglitazone, and could, potentially, attenuate some of the side effects of pioglitazone, particularly if a lower dose of the thiazolidinedione is

used. This review critically discusses the potential for early combination of pioglitazone and DPP4i.

Keywords: pioglitazone, DPP4i, combination, type 2 diabetes, cardiovascular outcomes

Introduction

Despite our increasing understanding of the pathogenesis of type 2 diabetes (T2DM) and the availability of new glucose-lowering agents, macrovascular complications and overall mortality associated with T2DM remains high.[1] Initial defect in insulin secretion and gradual loss of beta-cell function play an important role in the development of the disease. Many other factors (i.e., excessive glucagon release, impaired glucagon-like peptide-1 (GLP-1) secretion, augmented renal glucose reabsorption, and impaired central nervous system integration also contribute to the progression of the disease.[2]

T2DM, though heralded by hyperglycemia, is commonly associated with factors (e.g., central obesity, dyslipidemia, hypertension, and inflammation, among others) that increase risk of cardiovascular (CV) disease.[3] Epidemiological data shows a strong relationship between glucose levels and diabetes complications. Therefore, lowering HbA1c to as low and as safe a level as possible is a strategy proposed in most guidelines for optimizing diabetes care.[4]

Recent major T2DM trials have confirmed the importance of strict glycemic control to reduce the risk of microvascular complications [5] but have failed to demonstrate reductions in macrovascular events, suggesting that strategies taking into account global risk reduction rather than just focusing on lowering glucose levels are necessary. The Steno 2 trial [6] has shown how multifactorial intervention can, indeed, be very effective in T2DM patients reducing the relative risk of CV events by 51%. Primary intervention at an early stage of the natural history of diabetes could be even more effective since it has been calculated that just one-year delay in achieving good glycemic control (HbA1c <7.0%) can increase CV risk by approximately 60% as compared to patients achieving such a goal.[7]

Therefore, in the attempt to prevent the progression of the disease and reduce the risk of diabetic complications, early treatment should aim at ensuring durable glycemic control whilst conveying CV protection. The first goal requires tackling the main mechanisms underlying hyperglycemia, i.e., insulin resistance and beta-cell dysfunction, while, for the second goal, careful consideration of CV risk factors is paramount. To this purpose, ancillary properties of available glucose-lowering agents should be considered.

Metformin, the common front-line therapy in T2DM treatment, is considered an insulin sensitizer but pioglitazone exerts a stronger effect on insulin action in peripheral tissues.[8] Although metformin CV protection was apparent in the United Kingdom Prospective Diabetes Study (UKPDS) [9], more data lend support to the CV protection properties of pioglitazone.[10,11]

Among the agents used to improve beta-cell function, incretins have a more physiologic mechanism of action than, for instance, sulfonylureas (SU). Dipeptidyl peptidase-4 inhibitors (DPP4i) also have a very good safety and tolerability profile and, as such, they can be considered for combination with pioglitazone even in the early stage of the disease.

The purpose of this review article is to evaluate the potential of combination therapy with pioglitazone and DPP4i with respect to: (1) addressing pathophysiologic mechanisms underlying T2DM; (2) maintenance of sustained glycemic control; (3) effect on CV risk; and (4) overall safety.

Pathophysiologic-driven treatment of T2DM

Three major pathophysiologic mechanisms contribute to chronic hyperglycemia in T2DM: insulin resistance, progressive loss of beta-cell function, and excessive hepatic glucose output (HGO).

Loss of beta-cell function is key in determining the development and the progression of hyperglycemia in patients with T2DM as revealed in the UKPDS [12] and in the Belfast Diabetes Study.[13] The loss of beta-cell function occurs early in the natural history of T2DM. In the San Antonio Metabolism Study [14], subjects at high risk of developing T2DM with a 2-hour oral glucose tolerance test (OGTT) plasma glucose level in the high range of normal already had approximately 60% loss of beta-cell function. Inability to secrete timely and sufficient insulin in response to a stimulus is the result of a combination of impaired beta-cell function and beta-cell mass [15], both of which are believed to progressively decline over time contributing to the need of treatment escalation. Therefore, preserving beta-cell function is important for ensuring durable glycemic control.

Both DPP4i and pioglitazone have the potential to exert such an effect. Several studies in animals [16,17] have shown that DPP4i can preserve the histological architecture of the pancreatic islet as well as beta-cell mass and function in response to a number of stress conditions. This is believed to be the result of the persistence in the circulation of endogenously secreted GLP-1, a physiologic modulator of beta-cell mass, though a local, intra-islet GLP-1 release from alpha cells has been demonstrated in isolated human pancreatic islets.[18] The latter is of potential interest as dipeptidyl peptidase-4 is expressed in the pancreatic islets suggesting the existence of an intra-islet 'incretinergic' system that may contribute to beta-cell preservation. To what extent these mechanisms are active in T2DM patients is currently unclear, but DPP4i treatment has been shown to improve glucose sensitivity of the beta cell [19], i.e., the ability of the beta cell to sense and respond to changes in plasma glucose concentrations. However, data on the long-term effect of DPP4i on beta-cell function are lacking. More information will be generated with the

completion of the VERIFY (Vildagliptin Efficacy in combination with metfoRmIn For earlY treatment of T2DM) study (NCT01528254). In this trial, approximately 2,000, mainly drug-naïve, T2DM patients with a baseline HbA1c of 48–58 mmol/mol (6.5–7.5%) were randomized to either early initiation of a vildagliptin–metformin combination or standard-of-care initiation of metformin monotherapy, followed by stepwise addition of vildagliptin. The aim of this study is to determine treatment durability and changes in beta-cell function (HOMA-S) over a pre-specified 5-year follow-up.

In vivo and animal studies have provided evidence that glitazones also can exert a protective beta-cell effect.[20-22] Exposure of isolated human pancreatic islets to mild increase in free fatty acid (FFA) concentration is associated with inhibition of the expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) mRNA and impaired glucose-induced insulin secretion, a phenomenon practically reversed by rosiglitazone.[23] As discussed below, glitazones exert quite a durable effect with more patients sustaining good glycemic control over time. In the ADOPT trial, durability of rosiglitazone was associated not only with a significant improvement in insulin sensitivity, but also with a slower decline of beta-cell function.[24] Against this background, it seems rational to propose that pioglitazone and DPP4i may work through complementary mechanisms resulting in a more efficient beta-cell protection and, therefore, more sustained glycemic control.

The effect of the combination of pioglitazone and DPP4i on beta-cell function has been assessed in animal models as well as in human studies. In mutant obese (ob/ob) mice, the combined treatment exhibited a complementary effect, increasing plasma insulin levels by 3.2-fold and pancreatic insulin content by 2.2%).[25] Yin et al. [21] tested the ability of pioglitazone and alogliptin to enhance beta-cell regeneration of

endogenous and transplanted beta-cells in transgenic mice expressing firefly luciferase under the control of the mouse insulin-I promoter. Pioglitazone alone, or in combination with alogliptin, enhanced endogenous beta-cell regeneration in streptozotocin-treated mice. Moreover, while immunosuppression with rapamycin and tacrolimus caused early loss of beta-cell mass after islet transplantation, the use of pioglitazone and alogliptin partially promoted beta-cell mass recovery.[21]

The effect of the combination of the two agents on beta-cell function has been assessed in a 16-week study in 71 well-controlled T2DM patients (HbA1c 6.7 ± 0.1%) treated with alogliptin 25mg and piolitazone 30mg daily or daily alogliptin 25mg monotherapy or placebo.[26] The combination therapy improved beta-cell glucose sensitivity as well as fasting insulin secretion rate (vs. placebo; P=0.001), while alogliptin monotherapy had only slight, not significant, improvement of beta-cell function parameters.[26]

Insulin resistance is fully apparent in the pre-diabetic state [27] and it is responsible for impaired glucose utilization in insulin-dependent tissues (i.e., skeletal muscle, adipose tissue, and liver). Impaired insulin action can be exacerbated by concomitant obesity as the result of the excess of circulating free fatty acids (FFA), adipose-tissue mediated inflammatory cytokines (lipotoxicity), and infiltration of adipose tissue in the liver, muscle, and pancreas (ectopic fat). Defective insulin action and hyperglycemia can lead to changes in plasma lipoproteins [28] and the development of atherogenic dyslipidemia: elevated triglycerides, lowered HDL, and raised small, dense LDL.[28]

While no significant effect on insulin sensitivity is exerted by DPP4i, it is widely recognized that pioglitazone is a potent insulin sensitizer. This effect is associated with a reduction in serum levels of triglycerides and an increase of HDL-cholesterol

as a direct effect on apolipoprotein C-III (apoC3) and lipoprotein lipase activity.[29] Moreover, glitazones exert a powerful anti-inflammatory action.[30] The modulation of lipid metabolism and the anti-inflammatory property is the likely mechanism through which pioglitazone exerts powerful positive effects on nonalcoholic steatohepatitis (NASH).[31] The latter effect is of importance not only because of the potential evolution of NASH toward steatohepatitis, fibrosis, and hepato-carcinoma but also because NASH can contribute to inflammatory status and CV risk in T2DM.[32]

Treatment with glitazones is commonly associated with an increase in body weight. This is the result of a reduction of visceral fat at the expense of an increase in subcutaneous fat, a more benign fat tissue with milder metabolic implications.

Insulin resistance also accounts for excessive hepatic glucose production in the post-absorptive state and insufficient inhibition after the ingestion of a meal, thus contributing to both fasting and post-prandial hyperglycemia. Pioglitazone administration is associated with a significant reduction of liver glucose output.[33] The excess of glucose poured into the systemic circulation by the liver is mainly due to upregulated gluconeogenesis. The latter is the result of a complex and coordinated effect of multiple mechanisms including increased liver supply of gluconeogenic precursors (mainly lactate, pyruvate, alanine, and glycerol), allosteric activation of the initial gluconeogenic enzymes as a consequence of increased liver FFA oxidation [34], and inappropriately elevated portal concentration of glucagon. The increased flux of gluconeogenic precursors from the peripheral tissues is supported by impaired glucose oxidation with accumulation of pyruvate that becomes available for reduction to lactate and transamination to alanine.[35] Pioglitazone can reduce gluconeogenesis by ameliorating liver insulin sensitivity, enhancing peripheral glucose utilization and oxidation, and restraining lipolysis.

Of interest, DPP4i can reduce glucagon secretion [36] and, therefore, improve the insulin:glucagon molar ratio in the portal vein reducing hormonal activation of gluconeogenesis and hepatic glucose production. Moreover, experimental data suggest that DPP4i may directly affect liver glucose metabolism [37] therefore, even with respect to hepatic glucose production, pioglitazone and DPP4i can have a synergistic effect. In summary, the combination of pioglitazone and DPP4i addresses, in a synergistic manner, many of the pathogenic defects of T2DM by: (i) enhancing insulin secretion and suppressing glucagon release; (ii) improving incretin gut effects; (iii) enhancing insulin-mediated glucose utilization in peripheral tissues; (iv) restraining lipolysis; and (v) reducing gluconeogenesis.

Achieving long-lasting glycemic control

The effect of rosiglitazone, metformin, and glibenclamide as initial treatment was evaluated in 4,360 T2DM patients in the ADOPT trial.[38] After five years of treatment the cumulative incidence of monotherapy failure was 15% with rosiglitazone, 21% with metformin, and 34% with glyburide. The sustained efficacy of glitazones has been confirmed in many of the glitazone trials as summarized by DeFronzo and colleagues.[39] Similar results have been reported in an open-label, primary care observational study in 500 T2DM patients showing that pioglitazone, as an add-on to metformin, leads to significant benefits in long-term glycemic control compared with sulphonylureas.[40] In Japanese T2DM patients receiving pioglitazone, with or without other oral glucose-lowering drugs, better glycemic control was predicted to be maintained beyond the 2.5 to 4 years of observation.[41]

The longest randomized clinical trials with DPP4i run up to 2 years and compare glucose-lowering efficacy added-on to metformin (Met) vs. sulfonylureas. As shown

in Table 1, four out of 5 trials showed non-inferiority [42-45] and the fifth one was superior at the end of the second year.[46]

Clinical trials have directly explored the clinical efficacy of the DPP4i and pioglitazone association as initial combination therapy in drug-naive T2DM patients. Alogliptin (25 mg) and pioglitazone (30 mg) once daily for 26 weeks led to a greater HbA1c reduction (-1.7 ± 0.1%) than with alogliptin (-1.0 ± 0.1%; P<0.001) or pioglitazone (-1.2 ± 0.1%; P<0.001) monotherapy without worsening the respective safety profile.[47] Similar results have been reported with vildagliptin[48] and linagliptin.[49] In a 54-week randomized controlled extension trial, mean HbA1c reduction was -2.4% with the combination of sitagliptin 100 mg and pioglitazone 45 mg versus -1.9% with pioglitazone monotherapy and the mean reduction in fasting plasma glucose (FPG) was -61.3 mg/dl versus -52.8 mg/dl, with a comparable safety and tolerability of both treatment approaches.[50] Table 2 summarizes all clinical trials published in the past few years supporting the overall clinical efficacy of the treatment combination with pioglitazone and DPP4 inhibitors.

Pioglitazone, when added to metformin in T2DM patients failing with this treatment, was associated with a lower HbA1c reduction (-0.9 ± 0.05%) than adding pioglitazone plus alogliptin (-1.4 ± 0.05%; P <0.001) and associated with better proinsulin:insulin ratio and homeostasis model assessment of beta-cell function.[51] Moreover, 12-week treatment with sitaglitpin and pioglitazone enhanced the index Φ, a measure of dynamic β-cell responsiveness to glucose increments, to a greater extent than monotherapy versus placebo and versus either monotherapy alone.[52]

Altogether the results of these trials show how the combination of pioglitazone and DPP4i, two anti-hyperglycemic agents with different but complementary mechanisms

of action, provide a rational therapeutic approach in T2DM patients at different stages of the disease.

Treating both CVD and T2DM

Sustained glycemic control is key in reducing the risk of microvascular complications. Although, the impact of strict glycemic control on CV risk is still a matter of debate, preventing microvascular complications may exert a favorable effect on CV disease as well. Brownrigg et al.[53], by using a population-based cohort of T2DM patients, observed significant associations for a composite of CV events and retinopathy (Hazard Ratio (HR) 1·39, 95% CI 1·09-1·76), neuropathy (HR 1.40, 95% CI 1.19-1.66), and nephropathy (HR 1·35, 95% CI 1·15-1·58). Moreover, the presence of one, two, or three microvascular complications was associated with a worsening of HR by 1.32 (95% CI 1.16-1.50), 1.62 (95% CI 1.42-1.85), and 1.99 (95% CI 1.70-2.34) for CV risk, respectively.

Along with sustained glycemic control, pioglitazone conveys CV protection. In the PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) trial, adding pioglitazone to the existing therapy resulted in a non-significant 10% relative risk (RR) reduction in the primary composite endpoint of all-cause mortality, myocardial infarction (MI), acute coronary syndrome (ACS), cardiac intervention, stroke, major leg amputation and leg revascularization.[10] However, the risk reduction became significant when the pre-specified secondary endpoint of all-cause mortality, MI and stroke (HR 0.84, 95% CI 0.72–0.98; p=0.027) was considered.[54] This finding has been confirmed in post-hoc analyses showing reduction in recurrent myocardial infarction (HR 0.72, 95% CI 0.52–0.99; p=0.045) and stroke (HR 0.53, 95% CI 0.34–0.85; p=0.009)].[55,56] The latter finding set the basis for the Insulin Resistance Intervention after Stroke (IRIS) trial, exploring the effect of pioglitazone in insulin-

resistant, non-diabetic patients with a recent ischemic stroke or transient ischemic attack (TIA). The trial showed a 24% reduction in the risk of fatal or nonfatal stroke or myocardial infarction (HR 0.76; 95% CI, 0.62 - 0.93).[11] In a more recent meta-analysis including nine trials with 12,026 participants, pioglitazone was found to be associated with a lower risk of major adverse cardiovascular event (MACE) in patients with prediabetes or insulin resistance (RR 0.77, 95% CI 0.64 to 0.93), and diabetes (RR 0.83, 95% CI 0.72 to 0.97). Treatment with pioglitazone, however, was also associated with increased risk of heart failure (RR 1.32; CI 1.14 to 1.54).[57] The increased risk of HF with glitazones has widely been claimed as a consequence of fluid retention and edema formation attributed to salt-retaining effects of PPARγ activation on the nephron. However, in spite of a number of mechanisms responsible for fluid retention with thiazolidinediones (TZDs), there are few experimental and/or clinical studies that investigate the effects of TZDs on salt and water metabolism in patients with coronary heart disease (CHD).[58] Nonetheless, the effect of fluid retention as a worsening element for heart failure in T2DM patients should be taken into account particularly when considering the combination with DPP4i.

Recent CV safety trials with DPP4i found no reduction in CV death, [59-61] with the SAVOR-TIMI study, unexpectedly, reporting a significant increase in heart-failure hospitalizations with saxagliptin treatment ($p<0.007$).[59] This finding led to concerns about the potential link between DPP4i and heart failure. In the EXAMINE trial, hospital admission for heart failure was the first event in 85 (3·1%) patients taking aloglipitin compared with 79 (2·9%) taking placebo (HR 1·07, 95% CI 0·79–1·46).[62] In contrast, the TECOS trial reported no increase in hospitalization for heart failure.[61] Soon after the TECOS results were published, the FDA added a warning about the risk of heart failure on labels with the T2DM medicines saxagliptin and alogliptin.

However, whether individual DPP4i are associated with risk for HF is still a matter of debate.

A detailed look at SAVOR-TIMI found that patients with prior heart failure, higher levels of brain natriuretic peptide (BNP), and chronic kidney disease (eGFR ≤60ml/min) were at greatest risk for heart-failure hospitalization.[63] Patients in the high-risk EXAMINE trial with no baseline history of heart failure also had a significant increase in hospitalization for heart failure ($p<0.026$).[62] Each of the aforementioned trials is different and it would be difficult to compare them; hospital admission for heart failure in patients treated with DPP4i requires further study.

The only trial to look at the effect of DPP4i in heart failure patients with low left ventricular ejection fractions (LVEF) is the VIVIDD trial (Vildagliptin in Ventricular Dysfunction Diabetes Trial).[64] In this 52-week trial, 254 diabetes patients with systolic dysfunction (LVEF <35%) had a statistically significant increase in left ventricular end-diastolic volume (LVEDV), and a trend towards an increase in left ventricular end-systolic volume (LVESV). This increase in heart size with DPP4i is a concern and certainly warrants further investigation in patients with systolic dysfunction.

In summary, it remains unclear if DPP4i cause heart failure and, to add to the uncertainty, results from animal studies show improvement in left ventricular relaxation with the use of DPP4i. Moreover, a human trial using 3D echocardiography reported neutral results in diabetic patients with diastolic dysfunction treated with sitaglipitin.[65] One possible reason for this finding could be that there is no benefit or that it requires longer treatment in humans to determine either harm or benefit.

DPP4-inhibition may have a role in the progression of atherogenesis as suggested by recent animal research.[66] Additional studies have shown that elevated levels of DPP4 are present in insulin resistance states [67] and in patients with ACS.[68] This led to the hypothesis that the serine protease DPP4 plays an important role in the initiation and progression of atherosclerosis. Notably, DPP4 is a glycoprotein widely expressed in mammalian tissues and with more than 50 substrates, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Although DPP-4 inhibition can prevent the degradation of many peptides in *invitro* incubations, there is rather less convincing evidence that DPP-4 inhibition i*n vivo* actually increases levels of the endogenous peptide for many of these potential substrates. A study by Lee et al. found higher CD26/DPP4 levels in peripheral blood and T-cells in patients with T2DM.[69] Elevated DPP4 levels have also been found to cause insulin resistance at the level of protein kinase B (PKB; also known as AKT) phosphorlyation in fat cells, as well as in smooth and skeletal muscle.[70] Moreover, it should not be surprising that the toll-like receptor-4 (TLR-4), which is linked to atherosclerosis, is also affected by DPP4i. Ta et al. showed that alogliptin suppressed TLR-4, suggesting an important link with macrophage-mediated inflammation that is associated with tissue remodeling and atherosclerosis.[71] This basic research is clinically supported by the finding of reduced progression of carotid intima–media thickness (CIMT) with alogliptin in the recent human SPEAD-A (Study of Preventive Effects of Alogliptin on Diabetic Atherosclerosis) trial [72] With the exception of the PROLOGUE trial, [73] at least 3 other studies have shown potential anti-atherogenic effects of DPP4 inhibitors. Attenuation of CIMT progression has been observed with sitagliptin as an add-on to insulin treatment in T2DM patients free of apparent cardiovascular disease,[74] as well as in patients with impaired

glucose tolerance (IGT) or T2DM with stable angina pectoris.[75] Besides the potential direct effects of DPP4is on atherogenic mechanisms, reduction of glucose excursion, as achieved with DPP4i therapy, can also contribute to prevent CIMT progression.[76]

The effect of pioglitazone on atherosclerosis is more readily apparent. Two studies have demonstrated the beneficial impact of pioglitazone on the attenuation of atherosclerosis progression in T2DM patients as measured by carotid intima/medial thickness (CIMT) and coronary atheroma volume.[77] CHICAGO was a 72-week randomized, comparator-controlled trial that included 462 patients with T2DM.[78] This study demonstrated that CIMT progression was lower in the pioglitazone group compared to the glimepiride group (0.002mm vs. 0.026mm, respectively; P=0.008).[78] The PERISCOPE trial used intravascular ultrasound to look at atherosclerotic progression in 543 T2DM with coronary artery disease.[79] In the pioglitazone-treated group, plaque volume significantly decreased by 0.16%, whereas in patients receiving glimepiride, a mean increase of 0.73% was reported.[79]

Sustained increments in the serum triglyceride level are an independent risk factor for T2DM.[80] In the PERISCOPE trial, pioglitazone significantly increased high-density lipoprotein (HDL)-cholesterol levels and lowered triglycerides. Interestingly, a study by Nicholls et al. showed that the favorable effects of pioglitazone on the triglyceride/HDL-C ratio correlated with delayed atheroma progression in diabetic patients.[81]

In summary, several independent mechanisms may be activated by pioglitazone and DPP4i to support a complementary mechanism of action with the combination of the two medications in reducing the progression of atherosclerosis. Such an effect has received evidence as far as pioglitazone is concerned, whilst safety has been shown

for DPP4i. Therefore, in light of the need for early intervention with the purpose to achieve and maintain long-term glycemic control, the combination of the two agents can be seen as rational, also with regard to CV protection. Nonetheless, because of the increased risk of HF with pioglitazone and the concerns raised after the completion of the CV outcome trials with at least two out of the four DPP4i, a careful, balanced assessment of the risk to benefit ratio is recommended.

Safety considerations: the balance between risk and benefit

DPP4i alone or in combination with other antidiabetic drugs are generally well tolerated.[4] The risk of hypoglycemia is generally low[82] and mainly caused by concomitant insulin-delivery background therapy. In trials assessing the effect of DPP4i and pioglitazone, no significant increase in the rate of hypoglycemia has been reported. Therefore, to the extent that severe episodes of hypoglycemia may trigger CV events, the combination of the two drugs appears to be safe.

The increased risk of pancreatitis reported in early observational studies have not been confirmed in a number of investigations and a meta-analysis,[83,84] leading the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) to conclude there is no final evidence for a certain increase in the risk of pancreatitis or pancreatic neoplasia with the use of DPP4i.[85] The initial concern about bladder cancer with pioglitazone has also been dismissed in the light of the results of a prospective study, mandated by the FDA[86], and analyses of large databases.[87]

We have already discussed the relationship between DPP4i and risk of HF to conclude that it is difficult to determine whether this is a real phenomenon and/or specific to some DPP4i. Preclinical studies have identified a number of mechanisms that could actually suggest an improvement of heart function and *in vivo* studies, in addition to the results of SAVOR-TIMI and EXAMINE, have provided conflicting

results. For example, the analysis of the Italian Nationwide OsMed Health-DB Database has shown that in 127,555 T2DM patients, heart failure risk was lower in patients treated with DPP4i than in sulfonylurea-treated patients.[88] Nevertheless, careful assessment is required if a DPP4i combination with pioglitazone is considered, due to the common fluid retention associated with TZDs. In the PROactive trial, HF leading to hospital admission was more common in patients taking pioglitazone compared with placebo (5.7% vs. 4.1%). However, the HF-related mortality rate was lower with pioglitazone (26.8% vs. 34.3%).[10] It is worth considering that these studies have included patients with longstanding disease and high CV risk. Whether the same concern applies to patients at lower CV risk and at an earlier stage in the natural history of their disease remains to be established. Thus, although fluid retention can occur in 5% to 10% of glitazone-treated T2DM patients, less than 1% will develop HF. Moreover, a recent small clinical study using sophisticated measurement of heart function has suggested that pioglitazone can improve myocardial insulin sensitivity, LV diastolic function, and systolic function in T2D.[89] Improved myocardial insulin sensitivity and diastolic function are strongly correlated.[89]

Weight gain is the most common adverse effect associated with the use of glitazones due to fluid retention and increased adiposity. The latter, however, is associated with a relative redistribution of adipose tissue from visceral to subcutaneous stores.[90] DPP4i are usually neutral with respect to body weight and when used in combination with pioglitazone have resulted in either no change as compared to placebo [91] or slightly more weight gain compared with pioglitazone monotherapy.[92] Therefore, combination therapy with pioglitazone and DPP4i can be

expected to result in a mild, if any, increase in body weight in excess to the gain caused by pioglitazone itself.

Bone fractures are another potential side effect of pioglitazone treatment. These are mainly represented by low energy fractures (i.e., associated with a fall) of distal long bones of the upper and lower limbs, a finding recently confirmed in the IRIS population.[93] No signal for increased risk of bone fractures has been so far reported with the use of DPP4i, so no additional risk is expected when used in combination with pioglitazone. Actually, preclinical studies have suggested a protection of DPP4i on bone metabolism in animals treated with pioglitazone. The administration of vildagliptin to T2DM diabetic rats restored bone mass density, trabecular bone volume, and trabecular bone thickness, all parameters decreased by pioglitazone.[94] Also, fracture risk can be mitigated by fall prevention, screening and treatment of osteoporosis. Moreover, if CV risk were to be favorably affected by the combination treatment, this could outweigh the risk of fractures.

Most of the side effects associated with the use of pioglitazone, including the risk of bone fractures, appear to be dose dependent. Therefore, use of low doses of pioglitazone in combination with DPP4i may further reduce the risk of these side effects. In this regard, the effect of pioglitazone 7.5 mg/day as an add-on therapy in T2DM patients was compared to the 15mg and 30mg doses [95] showing that a significant increase in body weight and body fat was achieved with the two higher doses of pioglitazone but not the lowest one. Moreover, a significant reduction in triglyceride and increase in HDL cholesterol levels occurred in all three groups.

In summary, the combination of pioglitazone and DPP4i, as far as we can appreciate from the available data, is unlikely to exacerbate any of the known side effects

mainly due to pioglitazone. Actually, the concomitant use of DPP4i may attenuate some of these effects, particularly if a lower dose of pioglitazone is used.

Conclusions

In recent years, more emphasis has been being placed on earlier use of combination therapies for the treatment of T2DM.[96] Given the number of available drugs, there are quite a large number of potential combinations. Yet, combinations that may reduce chronic loss of beta-cell function, i.e., the main cause of the progression of the disease, while conferring CV protection may represent a preferred choice. Despite the fact that chronic hyperglycemia contributes and amplifies CV risk, a number of trials have failed to show a sizeable effect of intensive glycemic control.[97] Several trials have explored the CV safety of the glucose-lowering medications, with some of these trials showing significant reduction of CV risk. The first trial suggesting that mechanisms other than glucose could provide CV benefit was PROactive.[10] Although the trial did not meet the primary endpoint (due to the inclusion in the composite endpoint of revascularization; see Figure 1), pre-specified secondary endpoint and subsequent post-hoc analyses support a role for pioglitazone in reducing CV risk. The main secondary endpoint (i.e., cardiovascular death, non-fatal MI and stroke) was significantly reduced (HR 0.84; p=0.027) in PROactive. Of particular interest was the reduction of the risk for stroke that prompted the IRIS trial. The latter study has shown a 24% (P<0.007) reduction of the risk for fatal or nonfatal stroke or myocardial infarction in insulin-resistant individuals without diabetes and with a prior stroke.[11] In addition, a newly published secondary analysis from IRIS reported that pioglitazone reduced the risk of acute coronary syndrome (HR 0.71, 95% CI 0.54–0.94; P=0.02).[98] Moreover, there were significant reductions in the risk

for a type-1 MI (ST elevation MI) (HR 0.62, 95% CI 0.40–0.96) and risk of large MI (>100 troponin) >50% RR reduction (p<0.02; see Figure 2).[98]

With the addition of the TECOS trial results, DPP4i appear safe, in general, but a warning has been added to the United States prescribing information for saxagliptin and alogliptin informing physicians to consider the risks and benefits in patients at higher risk for heart failure. However, there is no apparent increased risk of heart failure when a broader population of T2DM patients is taken into account. Retrospective analysis found no increased risk of HF compared to sulfonylureas,[99] while a retrospective study based on the national Italian registry including 127,555 T2DM patients actually reported a reduction in the risk of hospitalization for HF as compared to sulfonylureas.[88] In the same population, no interclass difference was apparent for DPP4i with regard to the risk of hospitalization for HF.[100] These results, along with the overall tolerability profile, make DPP4i an attractive and safe treatment in the early stage of the disease. In patients with a longer duration of the disease and prior CV events or with high CV risk, DPP4i have been proven to be safe both in intervention trials,[59-61] as well as in population studies [101] and meta-analyses.[102] Caution may be used in those with a history of HF based on the selected DPP4i, paying attention to signs and symptoms of heart failure during therapy. In these individuals, concomitant use of a sodium-glucose co-transporter-2 (SGLT2) inhibitor may be also considered because of the reduced risk of HF and CV protection.[103]

Cardiology involvement in DPP4i is also important because of potential reductions in atherosclerosis and effects on myocardial remodeling. Extensive research is underway and further trials will help define their clinical use. Future basic and clinical studies will be required to determine the relative contribution of the non-enzymatic

vs. enzymatic molecular function in metabolic and inflammatory cardiovascular diseases, as well as to address HF safety signals and clarify a beneficial effect of this class in CV complications associated with diabetes.

Pioglitazone has been shown to have a durable glucose-lowering effect and a potential for preserving beta-cell function. DPP4i are characterized by sustained efficacy and have been shown to be safe with respect to CV risk, even in patients with recent ACS[60], i.e., patients at the highest risk so far studied with a DPP4i. These agents may also have a potential in preserving beta-cell function making a rational combination with pioglitazone while potentially attenuating some the side effects of the latter, particularly if lower doses of pioglitazone are used.

In summary, the rationale for combining a DPP4i and pioglitazone, particularly in the early stage of T2DM, is sound with respect to the pathophysiologic background of the disease, having potential for sustained glycemic control, and possibly conferring CV protection with an overall good safety and tolerability profile. The availability of fixed-dose combinations may also facilitate early introduction of this combination. Moreover, the combination of DPP4i and pioglitazone provides a useful example of what the diabetologist will have to do in the future, i.e., carefully weighing the pros and cons for each glucose-lowering drug. With therapeutic options expanding and with accumulating data with respect to CV safety and protection, the diabetologist will also have to identify rational and effective combination therapies that best suit individual needs to ensure durable glycemic control contributing to reduction of the risk of microvascular complications as well as to exploit extra-glycemic properties that may lower CV risk on an individual basis.

Acknowledgements

Klara Belzar (PhD) and XLR8 Health Ltd. UK, provided medical writing assistance for this manuscript.

References

1. Centers for Disease Control and Prevention. *National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States, 2011.* Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention;2011.
2. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care.* 2009;32 Suppl 2:S157-163.
3. Stolar MW, Chilton RJ. Type 2 diabetes, cardiovascular risk, and the link to insulin resistance. *Clin Ther.* 2003;25 Suppl B:B4-31.
4. Garber AJ, Abrahamson MJ, Barzilay JI, et al. Consensus Statement by the American Association of Clinical Endocrinologists and American College of Endocrinology on the Comprehensive Type 2 Diabetes Management Algorithm - 2017 Executive Summary. *Endocr Pract.* 2017.
5. Herrington WG, Preiss D. Tightening our understanding of intensive glycaemic control. *The Lancet Diabetes & Endocrinology.*
6. Gaede P, Oellgaard J, Carstensen B, et al. Years of life gained by multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: 21 years follow-up on the Steno-2 randomised trial. *Diabetologia.* 2016;59(11):2298-2307.
7. Paul SK, Klein K, Thorsted BL, Wolden ML, Khunti K. Delay in treatment intensification increases the risks of cardiovascular events in patients with type 2 diabetes. *Cardiovascular Diabetology.* 2015;14:100.
8. Natali A, Ferrannini E. Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: a systematic review. *Diabetologia.* 2006;49(3):434-441.

9. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 1998;352(9131):854-865.
10. Dormandy JA, Charbonnel B, Eckland DJ, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet.* 2005;366(9493):1279-1289.
11. Kernan WN, Viscoli CM, Furie KL, et al. Pioglitazone after Ischemic Stroke or Transient Ischemic Attack. *New England Journal of Medicine.* 2016;374(14):1321-1331.
12. Turner RC, Cull CA, Frighi V, Holman RR. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. *JAMA.* 1999;281(21):2005-2012.
13. Levy J, Atkinson AB, Bell PM, McCance DR, Hadden DR. Beta-cell deterioration determines the onset and rate of progression of secondary dietary failure in type 2 diabetes mellitus: the 10-year follow-up of the Belfast Diet Study. *Diabet Med.* 1998;15(4):290-296.
14. Gastaldelli A, Ferrannini E, Miyazaki Y, Matsuda M, DeFronzo RA. Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study. *Diabetologia.* 2004;47(1):31-39.
15. Cerf ME. Beta Cell Dysfunction and Insulin Resistance. *Frontiers in Endocrinology.* 2013;4:37.

16. Takeda Y, Fujita Y, Honjo J, et al. Reduction of both beta cell death and alpha cell proliferation by dipeptidyl peptidase-4 inhibition in a streptozotocin-induced model of diabetes in mice. *Diabetologia.* 2012;55(2):404-412.

17. Duttaroy A, Voelker F, Merriam K, et al. The DPP-4 inhibitor vildagliptin increases pancreatic beta cell mass in neonatal rats. *European journal of pharmacology.* 2011;650(2-3):703-707.

18. Marchetti P, Lupi R, Bugliani M, et al. A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. *Diabetologia.* 2012;55(12):3262-3272.

19. Mari A, Sallas WM, He YL, et al. Vildagliptin, a dipeptidyl peptidase-IV inhibitor, improves model-assessed beta-cell function in patients with type 2 diabetes. *J Clin Endocrinol Metab.* 2005;90(8):4888-4894.

20. Diani AR, Sawada G, Wyse B, Murray FT, Khan M. Pioglitazone preserves pancreatic islet structure and insulin secretory function in three murine models of type 2 diabetes. *Am J Physiol Endocrinol Metab.* 2004;286(1):E116-122.

21. Yin H, Park SY, Wang XJ, et al. Enhancing pancreatic Beta-cell regeneration in vivo with pioglitazone and alogliptin. *PLoS One.* 2013;8(6):e65777.

22. Ishida H, Takizawa M, Ozawa S, et al. Pioglitazone improves insulin secretory capacity and prevents the loss of beta-cell mass in obese diabetic db/db mice: Possible protection of beta cells from oxidative stress. *Metabolism: clinical and experimental.* 2004;53(4):488-494.

23. Lupi R, Del Guerra S, Marselli L, et al. Rosiglitazone prevents the impairment of human islet function induced by fatty acids: evidence for a role of PPARgamma2 in the modulation of insulin secretion. *Am J Physiol Endocrinol Metab.* 2004;286(4):E560-567.

24. Kahn SE, Lachin JM, Zinman B, et al. Effects of rosiglitazone, glyburide, and metformin on beta-cell function and insulin sensitivity in ADOPT. *Diabetes.* 2011;60(5):1552-1560.

25. Moritoh Y, Takeuchi K, Asakawa T, Kataoka O, Odaka H. The dipeptidyl peptidase-4 inhibitor alogliptin in combination with pioglitazone improves glycemic control, lipid profiles, and increases pancreatic insulin content in ob/ob mice. *European journal of pharmacology.* 2009;602(2-3):448-454.

26. Van Raalte DH, van Genugten RE, Eliasson B, et al. The effect of alogliptin and pioglitazone combination therapy on various aspects of beta-cell function in patients with recent-onset type 2 diabetes. *Eur J Endocrinol.* 2014;170(4):565-574.

27. Tabak AG, Herder C, Rathmann W, Brunner EJ, Kivimaki M. Prediabetes: a high-risk state for diabetes development. *Lancet.* 2012;379(9833):2279-2290.

28. Goldberg IJ. Diabetic Dyslipidemia: Causes and Consequences. *The Journal of Clinical Endocrinology & Metabolism.* 2001;86(3):965-971.

29. Ginsberg HN, Le NA, Goldberg IJ, et al. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. *The Journal of Clinical Investigation.* 1986;78(5):1287-1295.

30. Pfutzner A, Schondorf T, Hanefeld M, Forst T. High-sensitivity C-reactive protein predicts cardiovascular risk in diabetic and nondiabetic patients: effects of insulin-sensitizing treatment with pioglitazone. *Journal of diabetes science and technology.* 2010;4(3):706-716.

31. Gastaldelli A, Harrison S, Belfort-Aguiar R, et al. Pioglitazone in the treatment of NASH: the role of adiponectin. *Aliment Pharmacol Ther.* 2010;32(6):769-775.

32. Portillo-Sanchez P, Cusi K. Treatment of Nonalcoholic Fatty Liver Disease (NAFLD) in patients with Type 2 Diabetes Mellitus. *Clinical Diabetes and Endocrinology.* 2016;2(1):9.

33. Czaja MJ. Pioglitazone: More than just an Insulin Sensitizer. *Hepatology (Baltimore, Md).* 2009;49(5):1427-1430.

34. Laplante M, Festuccia WT, Soucy G, et al. Mechanisms of the depot specificity of peroxisome proliferator-activated receptor gamma action on adipose tissue metabolism. *Diabetes.* 2006;55(10):2771-2778.

35. Avogaro A, Toffolo G, Miola M, et al. Intracellular lactate- and pyruvate-interconversion rates are increased in muscle tissue of non-insulin-dependent diabetic individuals. *The Journal of Clinical Investigation.* 1996;98(1):108-115.

36. Balas B, Baig MR, Watson C, et al. The dipeptidyl peptidase IV inhibitor vildagliptin suppresses endogenous glucose production and enhances islet function after single-dose administration in type 2 diabetic patients. *J Clin Endocrinol Metab.* 2007;92(4):1249-1255.

37. Burcelin R, Gourdy P, Dalle S. GLP-1-based strategies: a physiological analysis of differential mode of action. *Physiology (Bethesda).* 2014;29(2):108-121.

38. Kahn SE, Haffner SM, Heise MA, et al. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med.* 2006;355(23):2427-2443.

39. DeFronzo RA, Eldor R, Abdul-Ghani M. Pathophysiologic approach to therapy in patients with newly diagnosed type 2 diabetes. *Diabetes Care.* 2013;36 Suppl 2:S127-138.

40. Hanefeld M, Pfutzner A, Forst T, Lubben G. Glycemic control and treatment failure with pioglitazone versus glibenclamide in type 2 diabetes mellitus: a 42-month, open-label, observational, primary care study. *Curr Med Res Opin.* 2006;22(6):1211-1215.

41. Stringer F, DeJongh J, Enya K, Koumura E, Danhof M, Kaku K. Evaluation of the long-term durability and glycemic control of fasting plasma glucose and glycosylated hemoglobin for pioglitazone in Japanese patients with type 2 diabetes. *Diabetes technology & therapeutics.* 2015;17(3):215-223.

42. Seck T, Nauck M, Sheng D, et al. Safety and efficacy of treatment with sitagliptin or glipizide in patients with type 2 diabetes inadequately controlled on metformin: a 2-year study. *Int J Clin Pract.* 2010;64(5):562-576.

43. Matthews DR, Dejager S, Ahren B, et al. Vildagliptin add-on to metformin produces similar efficacy and reduced hypoglycaemic risk compared with glimepiride, with no weight gain: results from a 2-year study. *Diabetes Obes Metab.* 2010;12(9):780-789.

44. Gallwitz B, Rosenstock J, Rauch T, et al. 2-year efficacy and safety of linagliptin compared with glimepiride in patients with type 2 diabetes inadequately controlled on metformin: a randomised, double-blind, non-inferiority trial. *Lancet.* 2012;380(9840):475-483.

45. Goke B, Gallwitz B, Eriksson JG, Hellqvist A, Gause-Nilsson I. Saxagliptin vs. glipizide as add-on therapy in patients with type 2 diabetes mellitus inadequately controlled on metformin alone: long-term (52-week) extension of a 52-week randomised controlled trial. *Int J Clin Pract.* 2013;67(4):307-316.

46. Del Prato S, Camisasca R, Wilson C, Fleck P. Durability of the efficacy and safety of alogliptin compared with glipizide in type 2 diabetes mellitus: a 2-year study. *Diabetes Obes Metab.* 2014;16(12):1239-1246.

47. Rosenstock J, Inzucchi SE, Seufert J, Fleck PR, Wilson CA, Mekki Q. Initial combination therapy with alogliptin and pioglitazone in drug-naive patients with type 2 diabetes. *Diabetes Care.* 2010;33(11):2406-2408.

48. Rosenstock J, Kim SW, Baron MA, et al. Efficacy and tolerability of initial combination therapy with vildagliptin and pioglitazone compared with component monotherapy in patients with type 2 diabetes. *Diabetes Obes Metab.* 2007;9(2):175-185.

49. Bajaj M, Gilman R, Patel S, Kempthorne-Rawson J, Lewis-D'Agostino D, Woerle HJ. Linagliptin improved glycaemic control without weight gain or hypoglycaemia in patients with type 2 diabetes inadequately controlled by a combination of metformin and pioglitazone: a 24-week randomized, double-blind study. *Diabet Med.* 2014;31(12):1505-1514.

50. Yoon KH, Steinberg H, Teng R, et al. Efficacy and safety of initial combination therapy with sitagliptin and pioglitazone in patients with type 2 diabetes: a 54-week study. *Diabetes, Obesity and Metabolism.* 2012;14(8):745-752.

51. DeFronzo RA, Burant CF, Fleck P, Wilson C, Mekki Q, Pratley RE. Efficacy and tolerability of the DPP-4 inhibitor alogliptin combined with pioglitazone, in metformin-treated patients with type 2 diabetes. *J Clin Endocrinol Metab.* 2012;97(5):1615-1622.

52. Alba M, Ahren B, Inzucchi SE, et al. Sitagliptin and pioglitazone provide complementary effects on postprandial glucose and pancreatic islet cell function. *Diabetes Obes Metab.* 2013;15(12):1101-1110.

53. Brownrigg JR, Hughes CO, Burleigh D, et al. Microvascular disease and risk of cardiovascular events among individuals with type 2 diabetes: a population-level cohort study. *Lancet Diabetes Endocrinol.* 2016;4(7):588-597.

54. Feng J, Zhang Z, Wallace MB, et al. Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV. *J Med Chem.* 2007;50(10):2297-2300.

55. Erdmann E, Dormandy JA, Charbonnel B, et al. The effect of pioglitazone on recurrent myocardial infarction in 2,445 patients with type 2 diabetes and previous myocardial infarction: results from the PROactive (PROactive 05) Study. *J Am Coll Cardiol.* 2007;49(17):1772-1780.

56. Wilcox R, Bousser MG, Betteridge DJ, et al. Effects of pioglitazone in patients with type 2 diabetes with or without previous stroke: results from PROactive (PROspective pioglitAzone Clinical Trial In macroVascular Events 04). *Stroke.* 2007;38(3):865-873.

57. Liao HW, Saver JL, Wu YL, Chen TH, Lee M, Ovbiagele B. Pioglitazone and cardiovascular outcomes in patients with insulin resistance, pre-diabetes and type 2 diabetes: a systematic review and meta-analysis. *BMJ Open.* 2017;7(1):e013927.

58. Goltsman I, Khoury EE, Winaver J, Abassi Z. Does Thiazolidinedione therapy exacerbate fluid retention in congestive heart failure? *Pharmacol Ther.* 2016;168:75-97.

59. Scirica BM, Bhatt DL, Braunwald E, et al. Saxagliptin and cardiovascular outcomes in patients with type 2 diabetes mellitus. *N Engl J Med.* 2013;369(14):1317-1326.

60. White WB, Bakris GL, Bergenstal RM, et al. EXamination of cArdiovascular outcoMes with alogliptIN versus standard of carE in patients with type 2 diabetes mellitus and acute coronary syndrome (EXAMINE): a cardiovascular safety study of the dipeptidyl peptidase 4 inhibitor alogliptin in patients with type 2 diabetes with acute coronary syndrome. *Am Heart J.* 2011;162(4):620-626 e621.

61. Green JB, Bethel MA, Armstrong PW, et al. Effect of Sitagliptin on Cardiovascular Outcomes in Type 2 Diabetes. *N Engl J Med.* 2015;373(3):232-242.

62. Zannad F, Cannon CP, Cushman WC, et al. Heart failure and mortality outcomes in patients with type 2 diabetes taking alogliptin versus placebo in EXAMINE: a multicentre, randomised, double-blind trial. *Lancet.* 2015;385(9982):2067-2076.

63. Scirica BM, Braunwald E, Raz I, et al. Heart failure, saxagliptin, and diabetes mellitus: observations from the SAVOR-TIMI 53 randomized trial. *Circulation.* 2014;130(18):1579-1588.

64. McMurray JJV, Ponikowski P, Bolli GB, et al. Effects of Vildagliptin on Ventricular Function in Patients With Type 2 Diabetes Mellitus and Heart Failure: A Randomized Placebo-Controlled Trial. *JACC Heart Fail.* 2017.

65. Oe H, Nakamura K, Kihara H, et al. Comparison of effects of sitagliptin and voglibose on left ventricular diastolic dysfunction in patients with type 2 diabetes: results of the 3D trial. *Cardiovascular Diabetology.* 2015;14(1):83.

66. Hirano T, Yamashita S, Takahashi M, Hashimoto H, Mori Y, Goto M. Anagliptin, a dipeptidyl peptidase-4 inhibitor, decreases macrophage infiltration

and suppresses atherosclerosis in aortic and coronary arteries in cholesterol-fed rabbits. *Metabolism: clinical and experimental.* 2016;65(6):893-903.

67. Sell H, Bluher M, Kloting N, et al. Adipose dipeptidyl peptidase-4 and obesity: correlation with insulin resistance and depot-specific release from adipose tissue in vivo and in vitro. *Diabetes Care.* 2013;36(12):4083-4090.
68. Yang G, Li Y, Cui L, et al. Increased Plasma Dipeptidyl Peptidase-4 Activities in Patients with Coronary Artery Disease. *PLoS One.* 2016;11(9):e0163027.
69. Lee SA, Kim YR, Yang EJ, et al. CD26/DPP4 levels in peripheral blood and T cells in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab.* 2013;98(6):2553-2561.
70. Lamers D, Famulla S, Wronkowitz N, et al. Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. *Diabetes.* 2011;60(7):1917-1925.
71. Ta NN, Li Y, Schuyler CA, Lopes-Virella MF, Huang Y. DPP-4 (CD26) inhibitor alogliptin inhibits TLR4-mediated ERK activation and ERK-dependent MMP-1 expression by U937 histiocytes. *Atherosclerosis.* 2010;213(2):429-435.
72. Mita T, Katakami N, Yoshii H, et al. Alogliptin, a Dipeptidyl Peptidase 4 Inhibitor, Prevents the Progression of Carotid Atherosclerosis in Patients With Type 2 Diabetes: The Study of Preventive Effects of Alogliptin on Diabetic Atherosclerosis (SPEAD-A). *Diabetes Care.* 2016;39(1):139-148.
73. Oyama J, Murohara T, Kitakaze M, et al. The Effect of Sitagliptin on Carotid Artery Atherosclerosis in Type 2 Diabetes: The PROLOGUE Randomized Controlled Trial. *PLoS Med.* 2016;13(6):e1002051.
74. Mita T, Katakami N, Shiraiwa T, et al. Sitagliptin Attenuates the Progression of Carotid Intima-Media Thickening in Insulin-Treated Patients With Type 2

Diabetes: The Sitagliptin Preventive Study of Intima-Media Thickness Evaluation (SPIKE): A Randomized Controlled Trial. *Diabetes Care.* 2016;39(3):455-464.

75. Ishikawa S, Shimano M, Watarai M, et al. Impact of sitagliptin on carotid intima-media thickness in patients with coronary artery disease and impaired glucose tolerance or mild diabetes mellitus. *Am J Cardiol.* 2014;114(3):384-388.
76. Barbieri M, Rizzo MR, Marfella R, et al. Decreased carotid atherosclerotic process by control of daily acute glucose fluctuations in diabetic patients treated by DPP-IV inhibitors. *Atherosclerosis.* 2013;227(2):349-354.
77. Pfutzner A, Marx N, Lubben G, et al. Improvement of cardiovascular risk markers by pioglitazone is independent from glycemic control: results from the pioneer study. *J Am Coll Cardiol.* 2005;45(12):1925-1931.
78. Mazzone T, Meyer PM, Feinstein SB, et al. Effect of pioglitazone compared with glimepiride on carotid intima-media thickness in type 2 diabetes: a randomized trial. *JAMA.* 2006;296(21):2572-2581.
79. Nissen SE, Nicholls SJ, Wolski K, et al. Comparison of pioglitazone vs glimepiride on progression of coronary atherosclerosis in patients with type 2 diabetes: the PERISCOPE randomized controlled trial. *JAMA.* 2008;299(13):1561-1573.
80. Beshara A, Cohen E, Goldberg E, Lilos P, Garty M, Krause I. Triglyceride levels and risk of type 2 diabetes mellitus: a longitudinal large study. *J Investig Med.* 2016;64(2):383-387.
81. Nicholls SJ, Tuzcu EM, Wolski K, et al. Lowering the triglyceride/high-density lipoprotein cholesterol ratio is associated with the beneficial impact of

pioglitazone on progression of coronary atherosclerosis in diabetic patients: insights from the PERISCOPE (Pioglitazone Effect on Regression of Intravascular Sonographic Coronary Obstruction Prospective Evaluation) study. *J Am Coll Cardiol.* 2011;57(2):153-159.

82. Foroutan N, Muratov S, Levine M. Safety and efficacy of dipeptidyl peptidase-4 inhibitors vs sulfonylurea in metformin-based combination therapy for type 2 diabetes mellitus: Systematic review and meta-analysis. *Clin Invest Med.* 2016;39(2):E48-62.
83. Monami M, Ahren B, Dicembrini I, Mannucci E. Dipeptidyl peptidase-4 inhibitors and cardiovascular risk: a meta-analysis of randomized clinical trials. *Diabetes Obes Metab.* 2013;15(2):112-120.
84. Azoulay L, Filion KB, Platt RW, et al. Association Between Incretin-Based Drugs and the Risk of Acute Pancreatitis. *JAMA Intern Med.* 2016;176(10):1464-1473.
85. Egan AG, Blind E, Dunder K, et al. Pancreatic safety of incretin-based drugs--FDA and EMA assessment. *N Engl J Med.* 2014;370(9):794-797.
86. Lewis JD, Habel LA, Quesenberry CP, et al. Pioglitazone Use and Risk of Bladder Cancer and Other Common Cancers in Persons With Diabetes. *JAMA.* 2015;314(3):265-277.
87. Levin D, Bell S, Sund R, et al. Pioglitazone and bladder cancer risk: a multipopulation pooled, cumulative exposure analysis. *Diabetologia.* 2015;58(3):493-504.
88. Fadini GP, Avogaro A, Degli Esposti L, et al. Risk of hospitalization for heart failure in patients with type 2 diabetes newly treated with DPP-4 inhibitors or other oral glucose-lowering medications: a retrospective registry study on

127,555 patients from the Nationwide OsMed Health-DB Database. *Eur Heart J.* 2015;36(36):2454-2462.

89. Clarke GD, Solis-Herrera C, Molina-Wilkins M, et al. Pioglitazone Improves Left Ventricular Diastolic Function in Subjects With Diabetes. *Diabetes Care.* 2017;40(11):1530-1536.
90. McLaughlin TM, Liu T, Yee G, et al. Pioglitazone increases the proportion of small cells in human abdominal subcutaneous adipose tissue. *Obesity (Silver Spring, Md).* 2010;18(5):926-931.
91. Rosenstock J, Brazg R, Andryuk PJ, Lu K, Stein P. Efficacy and safety of the dipeptidyl peptidase-4 inhibitor sitagliptin added to ongoing pioglitazone therapy in patients with type 2 diabetes: a 24-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. *Clin Ther.* 2006;28(10):1556-1568.
92. Garber AJ, Schweizer A, Baron MA, Rochotte E, Dejager S. Vildagliptin in combination with pioglitazone improves glycaemic control in patients with type 2 diabetes failing thiazolidinedione monotherapy: a randomized, placebo-controlled study*. *Diabetes, Obesity and Metabolism.* 2007;9(2):166-174.
93. Viscoli CM, Inzucchi SE, Young LH, et al. Pioglitazone and Risk for Bone Fracture: Safety Data from a Randomized Clinical Trial. *J Clin Endocrinol Metab.* 2016:jc20163237.
94. Eom YS, Gwon AR, Kwak KM, et al. Protective Effects of Vildagliptin against Pioglitazone-Induced Bone Loss in Type 2 Diabetic Rats. *PLoS One.* 2016;11(12):e0168569.
95. Rajagopalan S, Dutta P, Hota D, Bhansali A, Srinivasan A, Chakrabarti A. Effect of low dose pioglitazone on glycemic control and insulin resistance in

Type 2 diabetes: A randomized, double blind, clinical trial. *Diabetes Research and Clinical Practice.* 2015;109(3):e32-e35.

96. Bianchi C, Daniele G, Dardano A, Miccoli R, Del Prato S. Early Combination Therapy with Oral Glucose-Lowering Agents in Type 2 Diabetes. *Drugs.* 2017;77(3):247-264.

97. Turnbull FM, Abraira C, Anderson RJ, et al. Intensive glucose control and macrovascular outcomes in type 2 diabetes. *Diabetologia.* 2009;52(11):2288-2298.

98. Young LH, Viscoli CM, Curtis JP, et al. Cardiac Outcomes After Ischemic Stroke or Transient Ischemic Attack: Effects of Pioglitazone in Patients With Insulin Resistance Without Diabetes Mellitus. *Circulation.* 2017;135(20):1882-1893.

99. Kim YG, Yoon D, Park S, et al. Dipeptidyl Peptidase-4 Inhibitors and Risk of Heart Failure in Patients With Type 2 Diabetes Mellitus: A Population-Based Cohort Study. *Circ Heart Fail.* 2017;10(9).

100. Fadini GP, Saragoni S, Russo P, et al. Intraclass differences in the risk of hospitalization for heart failure among patients with type 2 diabetes initiating a dipeptidyl peptidase-4 inhibitor or a sulphonylurea: Results from the OsMed Health-DB registry. *Diabetes Obes Metab.* 2017;19(10):1416-1424.

101. Chan SY, Ou SM, Chen YT, Shih CJ. Effects of DPP-4 inhibitors on cardiovascular outcomes in patients with type 2 diabetes and end-stage renal disease. *Int J Cardiol.* 2016;218:170-175.

102. Xu S, Zhang X, Tang L, Zhang F, Tong N. Cardiovascular effects of dipeptidyl peptidase-4 inhibitor in diabetic patients with and without established

cardiovascular disease: a meta-analysis and systematic review. *Postgrad Med.* 2017;129(2):205-215.

103. Wilding JP, Rajeev SP, DeFronzo RA. Positioning SGLT2 Inhibitors/Incretin-Based Therapies in the Treatment Algorithm. *Diabetes Care.* 2016;39 Suppl 2:S154-164.

104. Pan C, Han P, Ji Q, et al. Efficacy and safety of alogliptin in patients with type 2 diabetes mellitus: A multicentre randomized double-blind placebo-controlled Phase 3 study in mainland China, Taiwan, and Hong Kong. *Journal of diabetes.* 2017;9(4):386-395.

105. Kaku K, Katou M, Igeta M, Ohira T, Sano H. Efficacy and safety of pioglitazone added to alogliptin in Japanese patients with type 2 diabetes mellitus: a multicentre, randomized, double-blind, parallel-group, comparative study. *Diabetes Obes Metab.* 2015;17(12):1198-1201.

106. Eliasson B, Moller-Goede D, Eeg-Olofsson K, et al. Lowering of postprandial lipids in individuals with type 2 diabetes treated with alogliptin and/or pioglitazone: a randomised double-blind placebo-controlled study. *Diabetologia.* 2012;55(4):915-925.

107. Henry RR, Staels B, Fonseca VA, et al. Efficacy and safety of initial combination treatment with sitagliptin and pioglitazone--a factorial study. *Diabetes Obes Metab.* 2014;16(3):223-230.

108. Derosa G, Cicero AF, Franzetti IG, et al. A randomized, double-blind, comparative therapy evaluating sitagliptin versus glibenclamide in type 2 diabetes patients already treated with pioglitazone and metformin: a 3-year study. *Diabetes technology & therapeutics.* 2013;15(3):214-222.

109. Yki-Jarvinen H, Rosenstock J, Duran-Garcia S, et al. Effects of adding linagliptin to basal insulin regimen for inadequately controlled type 2 diabetes: a >/=52-week randomized, double-blind study. *Diabetes Care.* 2013;36(12):3875-3881.

110. Kadowaki T, Kondo K. Efficacy and safety of teneligliptin in combination with pioglitazone in Japanese patients with type 2 diabetes mellitus. *J Diabetes Investig.* 2013;4(6):576-584.

111. Dormandy JA, Charbonnel B, Eckland DJ, et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet.* 2005;366(9493):1279-1289.

Table 1. Summary of the long-term (2 year) efficacy and safety trials of DPP4i added-on to metformin versus sulphonylureas in type 2 diabetes.

Author, year and reference	DPP4i	Comparator	Number of patients (n)	Baseline HbA1c % (mmol/mol)	ΔHbA1c (%) from baseline to 104 weeks	% Hypoglycemia	Primary endpoint outcome
Seck, 2010 [42]	sitagliptin	glipizide	504 PP (sitagliptin, n = 248; glipizide, n = 256)	7.3 (56) both groups	-0.54 sitagliptin and -0.51 glipizide	5% sitagliptin vs. 34% glipizide	Non-inferior
Matthews, 2010 [43]	vildagliptin	glimepiride	3118 randomized (vildagliptin, n = 1562; glimepiride, n = 1556)	7.3 (56) both groups	-0.1 both groups	vildagliptin 2.3% vs. 18.2% glimepiride	Non-inferior
Gallwitz, 2012 [44]	linagliptin	glimepiride	1519 PP (linagliptin, n = 764; glimepiride, n = 755)	7.7 (61) both groups	-0.16 linagliptin and -0·36 glimepiride	linagliptin 7% vs. 36% glimepiride	Non-inferior
Goke, 2013 [45]	saxagliptin	glipizide	858 randomized (saxagliptin, n= 428; glipizide, n = 430)	7.65 (60) both groups	-0.41 saxagliptin and -0.35 glipizide	saxagliptin 3.5% vs. 38.4% glipizide	Non-inferior
Del Prato, 2014 [46]	alogliptin	glipizide	1089 PP (alogliptin 12.5 mg once daily, n = 371; alogliptin 25 mg once daily, n = 382; and glipizide 5 mg once daily, n = 336)	7.6 (60) both groups	-0.68 alogliptin 12.5mg, -0.72 alogliptin 25mg, and -0.59 glipizide	2.5% and 1.4% alogliptin 12.5 and 25 mg, respectively vs. 23.2% glipizide	Superior in the alogliptin 25mg group

DPP4i: dipeptidyl peptidase-4 inhibitor; PP: per protocol

Table 2. Summary of recent clinical trials (last 5 years) evaluating the efficacy of the DPP4i and pioglitazone association in type 2 diabetes

Author, year and reference	DPP4i	Design	Subjects, n	Treatment arm and dose	Duration	HbA1c baseline %	Primary endpoint	Main results
Pan, 2017[104]	alogliptin	Multicenter, randomized DB, PBO, phase 3 study	506 T2DM	Patients were randomized 1:1 to receive: either 25 mg Alo once daily, or matching placebo. The groups were: (1) monotherapy (n = 185); (2) add-on to metformin (n = 197); and (3) add-on to Pio (with or without Met; n = 124)	16 weeks	entry criteria between 7.0% and 10.0%	Change from baseline HbA1c at Week 16	Alo add-on to either Met or Pio provided additional reduction in HbA1c at 16 weeks compared with placebo (-0.69 % [95% CI] -0.87%, -0.51%; P ≤ 0.001] and -0.52% [95% CI] -0.75%, -0.28%; P< 0.001], respectively.
Kaku, 2015[105]	alogliptin	Multicenter, randomized, DB, parallel group phase 4 study	210 T2DM	Patients were randomized 1:1:1 to receive: Alo 25mg/Pio 15 mg FDC, or Alo 25mg/Pio 30mg, or Alo 25 mg monotherapy	16 weeks	entry criteria between 6.5% and 10.5%	Change from baseline HbA1c at Week 16	FDC with Pio (15 mg and 30 mg) showed significant reduction in HbA1c than Alo monotherapy (-0.80 and -0.90% vs. 0%; p<0.0001, respectively).
Van Raalte, 2014 [26]	alogliptin	Two-center, randomized, DB, PBO, phase 3, parallel-arm intervention study	71 patients with well-controlled T2DM	Patients were randomized 1:1:1 to receive: Alo 25mg monotherapy q.d., or Alo 25mg/Pio 30mg FDC q.d., or placebo	16 weeks	6.7 ± 0.1% (SEM)	Change from baseline in postprandial incremental AUC for TG at Week 16	FPG was reduced to a greater extent by the Alo/Pio FDC compared with Alo monotherapy (P < 0.01).
Eliasson 2012	alogliptin	Two-center, randomized,	71 T2DM	Patients were	16 weeks	>6.5% at	Change from	Both Alo monotherapy

[106]		DB, PBO, parallel-group study	uncontrolled with lifestyle and/or Met, SU or glinide therapy	randomized 1:1:1 to receive: Alo 25mg monotherapy, or Alo 25mg/Pio 30mg FDC, or placebo		admission	baseline in postprandial incremental AUC for TG at Week 16	and Alo/Pio FDC treatment provided similar, statistically significant ($p<0.001$) reductions at week 16 in total postprandial TG compared with placebo; the Alo monotherapy group showed a greater trend to greater mean reduction compared to the Alo/Pio FDC group but this was not deemed statistically significant ($p=0.445$).
DeFronzo, 2012 [51]	alogliptin	Multicenter, randomized, DB, PBO, parallel-group study	1554 T2DM patients on stable-dose Met	Patients were randomized equally. The 12 treatment groups were: placebo, Alo monotherapy 12.5mg or 25mg oq.d., Pio monotherapy 15, 30, or 45mg q.d., Alo 12.5mg/Pio 15, 30, or 45mg FDC, and Alo 25mg/Pio 15, 30, or 45mg FDC	26 weeks	entry criteria between 7.5% and 10.0%	Change from baseline HbA1c at Week 26 or last observation	Added onto Met, the FDC Alo (12.5mg or 25mg)/Pio (15mg, 30mg or 45mg) once daily produced sustained and greater reductions in HbA1c compared to Pio monotherapy ($P<0.001$).
Henry, 2014[107]	sitagliptin	Randomized, factorial experimental study	1227 T2DM treatment-naïve patients	Patients were randomized to receive: q.d. either Sit 100mg monotherapy (n = 172), or Pio 15 (n = 163), 30 (n = 181) or 45mg (n = 171) monotherapy, or Sit 100mg plus Pio	54 weeks	entry criteria between 7.5% and 11.0%	Change from baseline HbA1c at Week 24	Initial combination therapy with Sit and Pio provided greater glycemic control than either monotherapy; significantly greater HbA1c reductions (0.4-0.7% difference).

				15 (n = 179), 30 (n = 173) or 45mg (n = 188) as initial therapy				
Derosa, 2013 [108]	sitagliptin	Randomized, DB, comparative study	436 overweight T2DM patients already treated with Pio and Met for 2 years completed the 3-year study	Patients were randomized to 1 year of Sit (n = 222) or glibenclamide (n = 214)	1 year treatment with Sit or glibenclamide	9.0% after 2-years run-in therapy augumenting phase with Met and Pio	Variation of beta-cell function both in a fasting stateand after euglycemic hyperinsulinemic and hyperglycemic clamp	Triple therapy with Sit greatly improved beta-cell function measures compared to glibenclamide, and also compared with the Met plus Pio dual combination.
Alba, 2013 [52]	sitagliptin	Randomized, PBO, observational study	211 T2DM patients	Patients were randomized 1:1:1:1 to Sit monotherapy, Pio monotherapy, Sit/Pio combination therapy, or placebo	12 weeks	between 6.5% and 9.0%	na	Sit/Pio combination enhances beta-cell function (increasing postmeal ϕ(s), a measure of dynamic beta-cell responsiveness to above-basal glucose concentrations) more than either monotherapy.
Yoon, 2012 [50]	sitagliptin	Randomized, DB, parallel-group extension study	317 treatment-naïve T2DM patients	Patients were randomized to initial Sit 100mg/Pio 30mg combination q.d. or Pio 30mg monotherapy q.d. for 24 weeks, Pio dose was increased from 30mg to 45mg in both groups in the extension study	54 weeks	between 8.0% and 12.0%	na	During the 54-week extension period, for the Sit/Pio combination the mean reduction in HbA1c was -2.4% with the Sit 100mg/Pio 45mg group vs. -1.9% with the Pio 45mg monotherapy group [between group difference (95% Cl) = -0.5% (-0.8, -0.3)],

								showing the combination led to substantial and durable incremental improvement in glycemic control compared to Pio monotherapy.
Bajaj, 2014 [49]	linagliptin	Multicenter, randomized, DB, PBO study	272 T2DM patients	Patients were randomized 2:1 to receive: either Lin 5mg q.d. or placebo, in addition to Met and Pio	24 weeks	between 7.5% and 10.0%	Change from baseline HbA1c at Week 24	Lin, as an add-on to Pio and Met, provided statistically significant and clinically meaningful reductions in HbA1c levels (change from baseline vs. placebo: (-0.57 (-0.13%); 95% Cl) -0.83, -0.31; P <0.0001).
Yki-jarvinen, 2013 [109]	linagliptin	Randomized, DB PBO study	1261 T2DM patients on basal insulin alone or combined with Met and/or Pio	Patients were randomized 1:1 to receive: either Lin 5mg q.d. (n = 631), or placebo (n = 630)	52 weeks	between 7.0% and 10.0%	Change from baseline HbA1c at Week 24	Lin, as an add-on to basal insulin (as well as Pio and Met), provided statistically significant and clinically meaningful reductions in HbA1c levels (change from baseline vs. placebo: (-0.7.1mmol/mol (-0.65%); 95% Cl) -0.74, -0.55; P <0.0001).
Kadowaki, 2013 [110]	teneligliptin	Randomized, DB, PBO, parallel-group study	204 T2DM patients taking Pio monotherapy	Patients were randomized 1:1 to receive: Ten 20mg q.d. or placebo q.d., as an add-on to stable Pio therapy (15 or 30mg q.d.)	12 weeks	between 6.8% and 10.3%	Change from baseline HbA1c at Week 12	Addition of Ten to Pio produced statistically significant and clinically meaningful reductions in HbA1c level compared to placebo (mean change from baseline to Week 52: -0.9% vs. -0.2%, respectively; P<0.001).

DB: double-blind; PBO: placebo-controlled; FDC: fixed dose combination; Alo: alogliptin; Pio: pioglitazone; Met: metformin; HbA1c: hemoglobin A1c; HDL: high-density lipoprotein cholesterol; AUC: area under curve; TG: triglycerides; SU: sulphonylurea; Sax: saxagliptin; TZD: thiazolidinedione; na: not applicable; q.d.: once daily; Lin: linagliptin; Ten: teneligliptin.

Figure 1. Original patient numbers for each component of the primary endpoint. It is of interest to note that all show less patient events with pioglitazone treatment, except for leg revascularization. Adapted from[111].

Figure 2. Secondary analysis from the IRIS trial finding important and significant reductions in acute coronary syndrome (ACS) patients receiving pioglitazone. The reduction in ACS, ST-Elevation Myocardial Infarction (STEMI) and risk of larger myocardial infarctions in patients with troponin >100 were all significant in 3,876 patients without diabetes that have insulin resistance. Adapted from[11].