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REVIEW ARTICLE

Practical strategies for improving outcomes in T2DM: The potential role of pioglitazone and DPP4 inhibitors

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T2DM is a complex disease underlined by multiple pathogenic defects responsible for the development and progression of hyperglycaemia. Each of these factors can now be tackled in a more targeted manner thanks to glucose-lowering drugs that have been made available in the past 2 to 3 decades. Recognition of the multiplicity of the mechanisms underlying hyperglycaemia calls for treatments that address more than 1 of these mechanisms, with more emphasis placed on the earlier use of combination therapies. Although chronic hyperglycaemia contributes to and amplifies cardiovascular risk, several trials have failed to show a marked effect from intensive glycaemic control. During the past 10 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-hyperglycaemic 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

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

1 | 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 remain 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 (ie, 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, although heralded by hyperglycaemia, is commonly associated with factors (eg, central obesity, dyslipidaemia, hypertension and inflammation, among others) that increase the 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 glycaemic 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 simply focusing on lowering glucose levels, are necessary. The Steno 2 trial[6] has shown how multifactorial

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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, as it has been calculated that even a 1-year delay in achieving good glycaemic control (HbA1c < 7.0%) can increase CV risk by approximately 60% as compared to patients achieving such a goal earlier.[7] Therefore, in the attempt to prevent progression of the disease and reduce the risk of diabetic complications, early treatment should aim at ensuring durable glycaemic control whilst conveying CV protection. The first goal requires tackling the main mechanisms underlying hyperglycaemia, that is, 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, 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 glycaemic control; (3) effect on CV risk; and (4) overall safety.

2 | PATHOPHYSIOLOGIC-DRIVEN TREATMENT OF T2DM

Three major pathophysiologic mechanisms contribute to chronic hyperglycaemia 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 hyperglycaemia 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 an 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 for treatment escalation. Therefore, preserving beta-cell function is important for ensuring durable glycaemic 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, although 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] that is, 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 2000, mainly drug-naïve, T2DM patients with a baseline HbA1c of 48 to 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 was 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 a 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 glycaemic control over time. In the ADOPT trial, the 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 more efficient beta-cell protection and, therefore, more sustained glycaemic 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 2 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 25 mg and pioglitazone 30 mg

daily or daily alogliptin 25 mg monotherapy or placebo.[26] The combination therapy improved beta-cell glucose sensitivity as well as the fasting insulin secretion rate (vs placebo; P = .001), while alogliptin monotherapy provided 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 (ie, 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 hyperglycaemia can lead to changes in plasma lipoproteins[28] and the development of atherogenic dyslipidaemia: 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 in 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 probable 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 in 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 hyperglycaemia. Pioglitazone administration is associated with a significant reduction in liver glucose output.[33] The excess of glucose poured into the systemic circulation by the liver is mainly the result of 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: (1) enhancing insulin secretion and suppressing glucagon release; (2) improving incretin gut effects; (3) enhancing insulin-mediated glucose utilization in peripheral tissues; (4) restraining lipolysis; and (5) reducing gluconeogenesis.

3 | ACHIEVING LONG-LASTING GLYCAEMIC CONTROL

The effect of rosiglitazone, metformin and glibenclamide as initial treatment was evaluated in 4360 T2DM patients in the ADOPT trial.[38] After 5 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 glycaemic control compared with sulphonylureas.[40] In Japanese T2DM patients receiving pioglitazone, with or without other oral glucose-lowering drugs, better glycaemic 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, 4 out of 5 trials showed non-inferiority[42–45] and the fifth trial 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 greater HbA1c reduction (−1.7% ± 0.1%) than that achieved with alogliptin (−1.0% ± 0.1%; P < .001) or pioglitazone (−1.2% ± 0.1%; P < .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 vs −1.9% with pioglitazone monotherapy, and the mean reduction in fasting plasma glucose (FPG) was −61.3 mg/dL vs −52.8 mg/dL, with comparable safety and tolerability using 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 of pioglitazone and DPP4 inhibitors.

Pioglitazone, when added to metformin in T2DM patients failing with this treatment, was associated with lower HbA1c reduction (−0.9% ± 0.05%) than that following addition of pioglitazone plus alogliptin (−1.4% ± 0.05%; P < .001) and with a better proinsulin: insulin ratio and better results of homeostasis model assessment of beta-cell function.[54] 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 vs placebo and vs either monotherapy alone.[57]

TABLE 1 Summary of the long-term (2 year) efficacy and safety trials of DPP4i added-on to metformin vs 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	% Hypoglycaemia	Primary endpoint outcome
Seck et al. 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 et al. 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 et al. 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 et al. 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 et al. 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 12.5 mg, −0.72 alogliptin 25 mg, and −0.59 glipizide	2.5% and 1.4% alogliptin 12.5 and 25 mg, respectively vs 23.2% glipizide	Superior in alogliptin 25 mg group

Abbreviations: DPP4i, dipeptidyl peptidase-4 inhibitor; PP, per protocol.

Altogether, the results of these trials show how the combination of pioglitazone and DPP4i, 2 anti-hyperglycaemic agents with different but complementary mechanisms of action, provide a rational therapeutic approach in T2DM patients at different stages of the disease.

4 | TREATING BOTH CVD AND T2DM

Sustained glycaemic control is key in reducing the risk of microvascular complications. Although the impact of strict glycaemic control on CV risk remains a matter of debate, preventing microvascular complications may exert a favourable effect on CV disease as well. Brownrigg et al.[60] 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 1, 2 or 3 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 glycaemic control, pioglitazone conveys CV protection. In the PROspective pioglitAzone Clinical Trial In macro-Vascular 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 = .027) was considered.[61] 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 = .045) and stroke (HR, 0.53; 95% CI, 0.34-0.85; P = .009)].[62,63] 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 9 trials with 12 026 participants, pioglitazone was found to be associated with lower risk of a major adverse cardiovascular event (MACE) in patients with prediabetes or insulin resistance (RR, 0.77; 95% CI, 0.64-0.93) and diabetes (RR, 0.83; 95% CI, 0.72-0.97). Treatment with pioglitazone, however, was also associated with increased risk of heart failure (RR, 1.32; 95% CI, 1.14-1.54).[64] The increased risk of HF with glitazones has been claimed widely as a consequence of fluid retention and edema formation attributed to the salt-retaining effects of PPARγ activation on the nephron. However, desspite a number of mechanisms responsible for fluid retention with thiazolidinediones (TZDs), there are few experimental and/or clinical studies that investigated the effects of TZDs on salt and water metabolism in patients with coronary heart disease (CHD).[65] Nonetheless, the effect of fluid retention as one worsening the risk of heart failure in T2DM patients should be taken into account, particularly when considering the combination with DPP4i.

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 et al. 2017[51]	Alogliptin	Multicentre, randomized DB, PBO, phase 3 study	506 T2DM	Patients randomized 1:1 to receive: either 25 mg Alo once daily, or matching placebo. 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%; $P \le .001$] and −0.52% [95% CI] −0.75%, −0.28%; $P < .001$], respectively
Kaku et al. 2015[52]	Alogliptin	Multicentre, randomized, DB, parallel group phase 4 study	210 T2DM	Patients randomized 1:1:1 to receive: Alo 25 mg/Pio 15 mg FDC, or Alo 25 mg/Pio 30 mg, 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 and 30 mg) showed significant reduction in HbA1c than Alo monotherapy (−0.80 and −0.90% vs. 0%; $P < .0001$, respectively)
Van Raalte et al. 2014[26]	Alogliptin	Two-centre, randomized, DB, PBO, phase 3, parallel-arm intervention study	71 patients with well-controlled T2DM	Patients randomized 1:1:1 to receive: Alo 25 mg monotherapy q.d., or Alo 25 mg/Pio 30 mg 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 ($P < .01$)
Eliasson et al. 2012[53]	Alogliptin	Two-centre, randomized, DB, PBO, parallel-group study	71 T2DM uncontrolled with lifestyle and/or Met, SU or glinide therapy	Patients randomized 1:1:1 to receive: Alo 25 mg monotherapy, or Alo 25 mg/Pio 30 mg FDC, or placebo	16 weeks	>6.5% at admission	Change from baseline in postprandial incremental AUC for TG at Week 16	Both Alo monotherapy and Alo/Pio FDC treatment provided similar, statistically significant ($P < .001$) 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 ($P = .445$).
DeFronzo et al. 2012[54]	Alogliptin	Multicentre, randomized, DB, PBO, parallel-group study	1554 T2DM patients on stable-dose Met	Patients randomized equally. The 12 treatment groups were: placebo, Alo monotherapy 12.5 or 25 mg oq.d., Pio monotherapy 15, 30, or 45 mg oq.d., Alo 12.5 mg/Pio 15, 30, or 45 mg FDC, and Alo 25 mg/Pio 15, 30, or 45 mg 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 (12.5 or 25 mg)/Pio (15, 30 or 45 mg) once daily produced sustained and greater reductions in HbA1c compared to Pio monotherapy ($P < .001$)

(Continues)

TABLE 2 (Continued)

Author, year and reference	DPP4i	Design	Subjects, n	Treatment arm and dose	Duration	HbA1c baseline %	Primary endpoint	Main results
Henry et al. 2014[55]	Sitagliptin	Randomized, factorial experimental study	1227 T2DM treatment-naïve patients	Patients randomized to receive: q.d. either Sit 100 mg monotherapy (n = 172), or Pio 15 (n = 163), 30 (n = 181) or 45 mg (n = 171) monotherapy, or Sit 100 mg plus Pio 15 (n = 179), 30 (n = 173) or 45 mg (n = 188) as initial therapy	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 glycaemic control than either monotherapy; significantly greater HbA1c reductions (0.4%-0.7% difference)
Derosa et al. 2013[56]	Sitagliptin	Randomized, DB, comparative study	436 overweight T2DM patients already treated with Pio and Met for 2 years completed the 3-year study	Patients 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 state and after euglycemic hyperinsulinemic and hyperglycaemic 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 et al. 2013[57]	Sitagliptin	Randomized, PBO, observational study	211 T2DM patients	Patients 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 et al. 2012[50]	Sitagliptin	Randomized, DB, parallel-group extension study	317 treatment-naïve T2DM patients	Patients randomized to initial Sit 100 mg/Pio 30 mg combination q.d. or Pio 30 mg monotherapy q.d. for 24 weeks, Pio dose was increased from 30 mg to 45 mg 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 100 mg/Pio 45 mg group vs. -1.9% with the Pio 45 mg monotherapy group [between group difference (95% CI) = −0.5% (−0.8, −0.3)], showing the combination led to substantial and durable incremental improvement in glycaemic control compared to Pio monotherapy

(Continues)

TABLE 2 (Continued)

Author, year and reference	DPP4i	Design	Subjects, n	Treatment arm and dose	Duration	HbA1c baseline %	Primary endpoint	Main results
Bajaj et al. 2014[49]	Linagliptin	Multicentre, randomized, DB, PBO study	272 T2DM patients	Patients randomized 2:1 to receive: either Lin 5 mg 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% CI) −0.83, −0.31; $P < .0001$)
Yki-jarvinen et al. 2013[58]	Linagliptin	Randomized, DB PBO study	1261 T2DM patients on basal insulin alone or combined with Met and/or Pio	Patients randomized 1:1 to receive: either Lin 5 mg 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: (−0.71 mmol/mol (−0.65%); 95% CI) −0.74, −0.55; $P < .0001$)
Kadowaki and Kondo, 2013[59]	Teneligliptin	Randomized, DB, PBO, parallel-group study	204 T2DM patients taking Pio monotherapy	Patients randomized 1:1 to receive: Ten 20 mg q.d. or placebo q.d., as an add-on to stable Pio therapy (15 or 30 mg 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; $P < .001$)

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

Recent CV safety trials with DPP4i found no reduction in CV death,[66–68] with the SAVOR-TIMI study, unexpectedly, reporting a significant increase in heart-failure hospitalizations with saxagliptin treatment ($P < .007$).[66] 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 alogliptin compared with 79 (2.9%) taking placebo (HR, 1.07; 95% CI, 0.79-1.46).[69] In contrast, the TECOS trial reported no increase in hospitalization for heart failure.[68] 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 of HF remains 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 ≤60 mL/min) were at greatest risk of heart-failure hospitalization.[70] Patients in the high-risk EXAMINE trial, with no baseline history of heart failure, also experienced a significant increase in hospitalization for heart failure ($P < .026$).[69] 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).[71] In this 52-week trial, 254 diabetes patients with systolic dysfunction (LVEF <35%) experienced 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 whether 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.[72] A 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.[73] Additional studies have shown that elevated levels of DPP4 are present in insulin resistance states[74] and in patients with ACS.[75] 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, with more than 50 substrates, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). Although DPP4 inhibition can prevent the degradation of many peptides in in vitro incubations, there is rather less convincing evidence that DPP4 inhibition in 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.[76] 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.[77] 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.[78] 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.[79] With the exception of the PROLOGUE trial,[80] 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,[81] as well as in patients with impaired glucose tolerance (IGT) or T2DM with stable angina pectoris.[82] Besides the potential direct effects of DPP4i inhibitors on atherogenic mechanisms, reduction of glucose excursion, as achieved with DPP4i therapy, can also contribute to prevention of CIMT progression.[83]

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.[84] CHICAGO was a 72-week randomized, comparator-controlled trial that included 462 patients with T2DM.[85] This study demonstrated that CIMT progression was lower in the pioglitazone group compared to the glimepiride group (0.002 mm vs 0.026 mm, respectively; $P = .008$).[85] The PERISCOPE trial used intravascular ultrasound to look at atherosclerotic progression in 543 T2DM patients with coronary artery disease.[86] 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.[86]

Sustained increments in serum triglyceride level are an independent risk factor for T2DM.[87] 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 favourable effects of pioglitazone on the triglyceride/HDL-C ratio correlated with delayed atheroma progression in diabetic patients.[88]

In conclusion, several independent mechanisms may be activated by pioglitazone and DPP4i to support a complementary mechanism of action with the combination of the 2 medications in reducing the progression of atherosclerosis. There is evidence of such an effect 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 of achieving and maintaining long-term glycaemic control, the combination of the 2 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 2 out of the 4 DPP4i, a careful, balanced assessment of the risk-to-benefit ratio is recommended.

5 | SAFETY CONSIDERATIONS: BALANCE BETWEEN RISK AND BENEFIT

DPP4i alone or in combination with other antidiabetic drugs are generally well tolerated.[4] The risk of hypoglycaemia is generally low[89]

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 hypoglycaemia has been reported. Therefore, to the extent that severe episodes of hypoglycaemia 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,[90,91] leading the Food and Drug Administration (FDA) and the European Medicine Agency (EMA) to conclude that there is no final evidence for a certain increase in the risk of pancreatitis or pancreatic neoplasia with the use of DPP4i.[92] The initial concern about bladder cancer with use of pioglitazone has also been dismissed, in light of the results of a prospective study, mandated by the FDA,[93] and analyses of large databases.[94]

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, analysis of the Italian Nationwide OsMed Health-DB Database has shown that, in 127 555 T2DM patients, the risk of heart failure was lower in patients treated with DPP4i than in sulfonylurea-treated patients.[95] Nevertheless, careful assessment is required if a DPP4i combination with pioglitazone is considered, because of the common fluid retention associated with TZDs. In the PROactive trial, HF leading to hospital admission was more common in patients using 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 risk of CV and at an earlier stage in the natural history of the 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.[96] Improved myocardial insulin sensitivity and diastolic function are strongly correlated.[96]

Weight gain is the most common adverse effect associated with the use of glitazones because of fluid retention and increased adiposity. The latter, however, is associated with a relative redistribution of adipose tissue from visceral to subcutaneous stores.[97] 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[98] or slightly more weight gain compared with pioglitazone monotherapy.[99] Therefore, combination therapy with pioglitazone and DPP4i can be expected to result in a mild, if any, increase in body weight in excess of the gain caused by pioglitazone itself.

Bone fractures are another potential side effect of pioglitazone treatment. These are mainly low energy fractures (ie, associated with a fall) of distal long bones of the upper and lower limbs, a finding recently confirmed in the IRIS population.[100] No signal for increased risk of bone fractures has been reported with the use of DPP4i; thus, no additional risk is expected when used in combination with pioglitazone. Actually, preclinical studies have suggested a protective effect 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.[101] Also, the risk of fracture can be mitigated by fall prevention and by screening and treatment of osteoporosis. Moreover, if CV risk were to be favourably 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 15 and 30 mg doses,[102] and showed that a significant increase in body weight and body fat was achieved with the 2 higher doses of pioglitazone, but not with the lowest dose. Moreover, a significant reduction in triglyceride and an increase in HDL cholesterol levels occurred in all 3 groups.

In summary, the combination of pioglitazone and DPP4i, as far as we can determine from the available data, is unlikely to exacerbate any of the known side effects mainly related to pioglitazone. Actually, the concomitant use of DPP4i may attenuate some of these effects, particularly if a lower dose of pioglitazone is used.

6 | CONCLUSIONS

In recent years, more emphasis has been placed on earlier use of combination therapies for the treatment of T2DM.[103] Given the number of available drugs, there is quite a large number of potential combinations. Yet, combinations that may reduce chronic loss of beta-cell function, that is, the main cause of the progression of the disease, while conferring CV protection, may be a preferred choice. Despite the fact that chronic hyperglycaemia contributes to and amplifies CV risk, a number of trials have failed to show a sizeable effect of intensive glycaemic control.[104]

Several trials have explored the CV safety of the glucose-lowering medications, with some of these trials showing significant reduction in CV risk. The first trial to suggest that mechanisms other than glucose could provide CV benefit was PROactive.[10] Although the trial did not meet the primary endpoint (because of inclusion in the composite endpoint of revascularization) (Figure 1), the pre-specified secondary endpoint and subsequent post-hoc analyses support a role for pioglitazone in reducing CV risk. The main secondary endpoint (ie, cardiovascular death, non-fatal MI and stroke) was significantly reduced (HR, 0.84; P = .027) in PROactive. Of particular interest was the reduction in the risk of stroke that prompted the IRIS trial. The latter study showed a 24% (P < .007) reduction in the risk of 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,
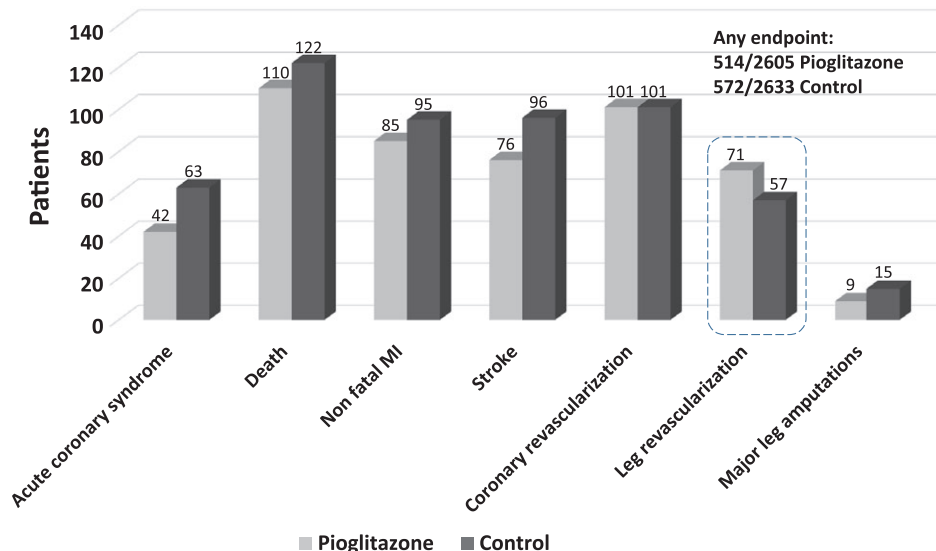
FIGURE 1 Original patient numbers for each component of the primary endpoint. It is of interest to note that all show less patient events with pioglitazone treatment, with the exception of leg revascularization. Adapted from [10]

0.71; 95% CI, 0.54-0.94; P = .02).[105] Moreover, there were significant reductions in the risk of a type-1 MI (ST elevation MI) (HR, 0.62; 95% CI, 0.40-0.96) and the risk of large MI (>100 troponin) > 50% RR reduction (P < .02) (Figure 2).[105]

With the addition of the TECOS trial results, DPP4i appear to be safe, in general, but a warning has been added to US prescribing information for saxagliptin and alogliptin, informing physicians to consider the risks and benefits in patients at higher risk of 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 use of sulfonylureas,[106] 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 that with use of sulfonylureas.[95] In the same population, no interclass difference was apparent for DPP4i with regard to the risk of hospitalization for HF.[107] 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 in intervention trials,[66–68] as well as in population studies[108] and meta-analyses.[109] Caution may be used in individuals 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 considered also because of the reduced risk of HF and CV protection.[110]

Cardiology involvement in DPP4i is 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, and 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,[67] that is, patients at the highest risk so far studied with a DPP4i. These agents may also have potential in preserving beta-cell function, making a rational combination with pioglitazone while potentially attenuating some of the side effects of the latter, particularly if lower doses of pioglitazone are used.

In summary, the rationale for combining 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 glycaemic control, and possibly conferring CV protection with
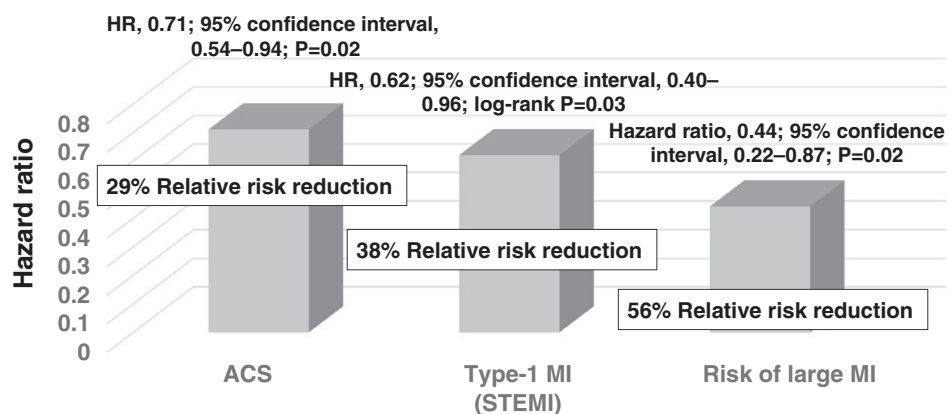

FIGURE 2 Secondary analysis from the IRIS trial finding important and significant reductions in acute coronary syndrome (ACS) patients receiving pioglitazone. The reduction in ACS, ST-Elevation Myocardial Infarction (STEMI) and risk of larger myocardial infarctions in patients with troponin >100 were all significant in 3876 patients without diabetes that have insulin resistance. Adapted from [105]

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 must do in the future, that is, carefully weigh the pros and cons of each glucose-lowering drug. With therapeutic options expanding, and with accumulating data with respect to CV safety and protection, the diabetologist must also identify rational and effective combination therapies that best suit individual needs, to ensure durable glycaemic control that contributes to reduction of the risk of microvascular complications, and to exploit extra-glycaemic properties that may lower CV risk on an individual basis.

Conflict of interest

S. D. P. has received research support from Astra Zeneca, Boehringer Ingelheim, Merck & Co. and Novartis Pharmaceuticals, and has served on advisory panels for Astra Zeneca, Boehringer Ingelheim, Eli Lily and Co., GlaxoSmithKline, Janssen Pharmaceuticals, Merck & Co. and Novartis Pharmaceuticals, Novo Nordisk, Sanofi, Laboratoires Servier and Takeda Pharmaceuticals. R. C. has received research support from Boehringer Ingelheim, Takeda Pharmaceuticals and Merck Sharp & Dohme (MSD).

Author contributions

S. D. P. and R. C. have equally contributed to searching literature, summarizing and critically nalysing results, and riting and finalizing the present work.

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