REVIEW

Diabetes Management

An overview of non-insulin adjunctive therapies for Type 1 diabetes

Chitrabhanu Ballav*[1] & Stephen C L Gough[1]

Practice points

- Most patients with type 1 diabetes on exogenous insulin injections do not achieve glycaemic goals
- Exogenous insulin treatment has limitations including, insulin related weight gain and recurrent hypoglycaemia
- Evidence suggest insulin microsecretion from functioning ß-cells long after diagnosis of type 1 diabetes
- Almost all patients with type 1 diabetes have inappropriately high glucagon levels, which contribute to hyperglycaemia
- Reduced insulin sensitivity increases insulin dose requirement
- Metformin improves organ sensitivity to insulin and may have a role in enhancing insulin secretion
- Clinical trials suggest modest benefit in glycaemic control with metformin
- There is a possible role for metformin in reducing risk of cardiovascular disease in type 1 diabetes
- Thiazolidinediones improve organ sensitivity to insulin and may have immunogenic role
- Clinical trials with thiazolidinediones suggest small benefit on glycaemic control in type 1 diabetes
- α-glucosidase inhibitors reduce glucose absorption from gut
- Small study suggests limited benefit in glycaemic control
- Clinical trials suggest that amylin analogues have some benefit in controlling post-prandial hyperglycaemia
- Amylin analogues require frequent dosing with meals reducing clinical utility
- Treatment targeting GLP-1 receptors aim to stimulate insulin and suppress glucagon levels
- Clinical studies on GLP-1 receptor agonists suggest possible benefit on post-prandial hyperglycaemia and overall insulin requirement
- Small studies suggest a role for GLP-1 agonists in supporting declining graft function in recipients of islet or pancreatic transplant
- DPP-4 inhibitors increase endogenous GLP-1 levels and there is modest benefit on glycaemic control in short term studies
- Leptin probably has a role in glucagon suppression and may be useful in type 1 diabetes patients, but clinical trials are needed
- Immune prevention strategies have used low dose insulin to prevent diabetes in patients at high risk of type 1 diabetes, with little overall benefit
- Non-antigen specific immunosuppression may prolong remission from type 1 diabetes but there are concerns over toxicity
- Current research strategies are looking at expanding numbers of regulator T cells as a way of targeting specific auto-antigens while sparing the remaining immune system.

[1]NIHR Oxford Biomedical Research Centre & Oxford Centre for Diabetes Endocrinology and Metabolism, Oxford University Hospitals NHS Trust, Oxford, OX3 7LE, UK
*Author for correspondence: Tel.: +44 1865 857117; Fax: +44 1865 857074; chitrabhanu.ballav@hmc.ox.ac.uk

Future Medicine part of fsg

KEYWORDS
• amylin • hypoglycemia
• glucagon • incretins
• leptin • metformin
• Type 1 diabetes

ABSTRACT Most people with Type 1 diabetes are managed by insulin injections, with many not achieving glycemic targets, placing them at an increased risk of complications. The reasons behind suboptimal control are numerous but include limitations of insulin therapy. People with Type 1 diabetes may have residual β-cell function for many years after diagnosis. Almost all have inappropriate glucagon release contributing to poor glycemic control. Agents that preserve or enhance β-cell function along with therapies that improve α-cell function, glucagon regulation and insulin sensitivity are currently being actively evaluated. This review focuses on agents that in combination with insulin are likely to have a beneficial role in the prevention and progression of Type 1 diabetes and the optimization of medical care.

Autoimmune destruction of the β cells in the islets of Langerhans of the pancreatic islets results a life-long dependence on exogenous insulin for people with Type 1 diabetes mellitus.

Over the last few decades there have been significant advances in insulin replacement therapy, leading to the development of both short- and long-acting insulin analog preparations. These insulins allow administration in a manner that allows people with Type 1 diabetes to more closely mimic physiological insulin profiles. Insulin delivery technology has also come a long way with the replacement of ampules and syringes by 'pen' systems and a range of insulin pumps including those in which insulin can be delivered in response to glucose, measured in real-time. Despite these developments, glycemic management in patients with Type 1 diabetes remains suboptimal. In 2012, only approximately 20% of people with Type 1 diabetes in a UK study had an HbA1C <7.5% (58 mmol/mol) [1]. It is becoming increasingly recognized that insulin treatment alone may not be sufficient, certainly in the long-term, to manage blood glucose levels, leading to an increasing interest in additional, non-insulin adjunct, treatments for people with Type 1 diabetes.

One of the major limiting factors to achieving near normal blood glucose levels with exogenous insulin replacement is unwanted and indeed unpredictable low blood glucose values or hypoglycemia [2]. Patients with Type 1 diabetes have around two episodes of symptomatic hypoglycemia per week. The incidence of severe hypoglycemia, defined as requiring help for recovery from a third party, has a prevalence of 30–40% per year and an incidence of 1–1.7 episodes per patient per year [3,4]. The factors contributing to hypoglycemia in Type 1 diabetes include systemic hyperinsulinemia that fails to respond to falling blood glucose, an inability to mount an adequate counter-regulatory glucagon response, intraislet sympathetic neuropathy, and a reduced autonomic response, which in turn impairs release of glucose from the liver at low blood glucose, delaying recovery [3].

Why might non-insulin adjuncts be of benefit to people with Type 1 diabetes?

In every day clinical practice it is clear that some people with Type 1 diabetes have more troublesome hypoglycemia than others. Interestingly, recent evidence suggests insulin deficiency in Type 1 diabetes is not absolute, implying residual β-cell function, in some subjects [5–7]. In a study of patients being treated for Type 1 diabetes for more than 50 years more than 67% were found to have a random C peptide level >30 pM [6]. Using an ultrasensitive assay, further data suggest more than 70% of patients being treated for Type 1 diabetes had fasting C peptide more than 3.3 pM after a median duration of 30 years from diagnosis [7]. There was also an increase in C peptide in response to mixed meal in almost 80% of these patients. Histological sections of pancreas tissue from patients treated for Type 1 diabetes for a long duration of 63 years also revealed the presence of surviving β cells [6,8]. Persistence of C peptide production has been found to have a protective effect on development of microvascular complications. Patients with some endogenous insulin production also have lower rates of hypoglycemia [9]. Therapeutic strategies that aim to preserve or even enhance β-cell function are likely to be of benefit, therefore, in the management of Type 1 diabetes.

It is also becoming increasingly evident that insulin resistance plays an important role in the 'progression' of Type 1 diabetes. Improvement in insulin sensitivity at around 3 months after starting insulin in newly diagnosed Type 1 patients is likely to contribute to the transient remission seen during the 'honeymoon period'. However, as Type 1 diabetes becomes more long-standing, insulin sensitivity appears to decline. Insulin sensitivity has been reported to be at

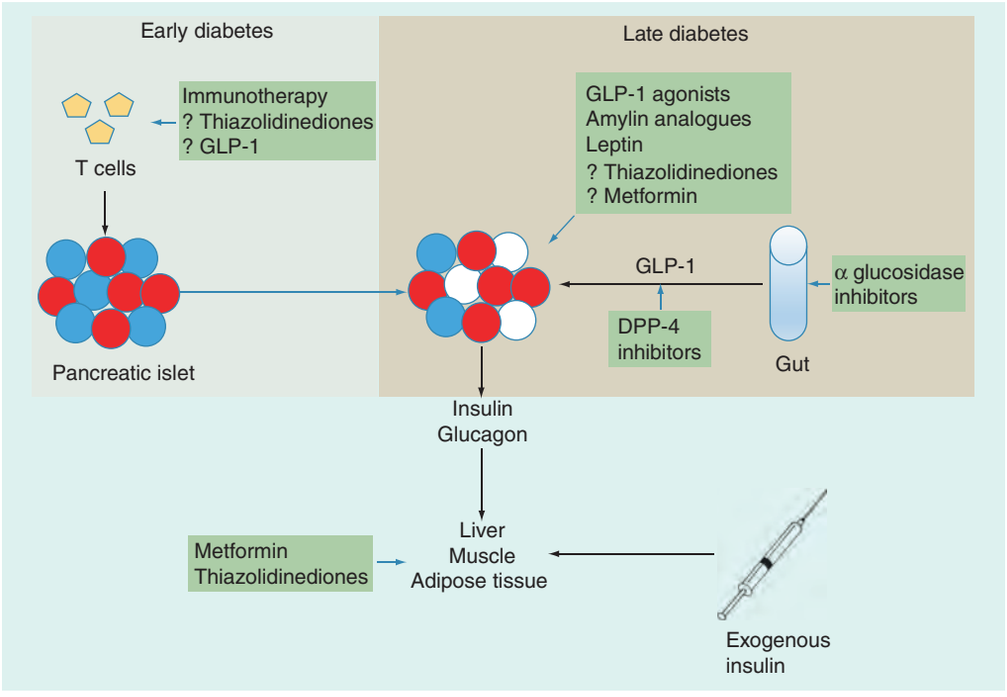

Figure 1. Targets for non-insulin treatment in early and late Type 1 diabetes. The targets for non-insulin adjunct treatment at various points in the time course of Type 1 diabetes. Pancreatic islets (red circles: for α cells; blue circles: for β cells) are attacked by the autoimmune process, which may be delayed/partially reversed by immunotherapy and also with a possible role for thiazolidinediones (TZD) and GLP-1 receptor agonists. Later in diabetes GLP-1 receptor agonists, amylin analogs and leptin may work on pancreatic islets to stimulate residual insulin secretion and/or suppress glucagon secretion. Roles for TZDs and metformin have been suggested but need to be confirmed in clinical trials. Metabolic regulators at other stages of diabetes include, for example, DPP-4 inhibitors, which increase endogenous GLP-1 levels, α-glucosidase inhibitors, which reduce carbohydrate absorption, and metformin and TZDs, which increase organ sensitivity to insulin. Question marks indicate treatments not as firmly established as those without questions marks.

approximately 60% of that in people without diabetes after 10 years of diagnosis of Type 1 diabetes [10,11]. Treatment aimed at improving insulin sensitivity may, therefore, also be beneficial especially in recent onset Type 1 diabetes before 'resistance' becomes established.

Finally, the role of glucagon in Type 1 diabetes has been the focus of renewed attention, with elevations in both fasting and postprandial glucagon values. Plasma glucagon levels have been found to be raised and to correlate with hyperglycemia in patients presenting with diabetic ketoacidosis [12]. Suppression of glucagon with somatostatin has been reported to prevent the development of ketoacidosis in patients following withdrawal of insulin treatment [13]. Recent evidence suggests that in Type 1 diabetes glucagon levels after a mixed meal are high as early as the first year of diagnosis and is associated with postprandial hyperglycemia [14]. As mentioned before, in people with Type 1 diabetes, glucagon levels fail to rise during hypoglycemia, thereby impairing recovery. The abnormal glucagon response appears to be mainly because of the effect of systemic hyperinsulinemia on glucagon secretion working through central and peripheral mechanisms [15,16]. Pharmacological agents capable of regulating glucagon secretion in a glucose-dependent manner may help address inappropriately high glucagon after meals and inappropriately low levels during hypoglycemia.

The article will now provide an overview of how some of these relatively recent metabolic observations in Type 1 diabetes may pave the way for the use of non-insulin adjuncts as a treatment for some people with Type 1 diabetes. The possible targets for such interventions are

outlined in **Figure 1**. Immunotherapy for Type 1 diabetes is another area where there have been rapid advances. While we discuss this briefly, this topic is beyond the scope of the current review.

Metformin

The biguanide, metformin is frequently used as first-line therapy in people with Type 2 diabetes. In hepatocytes metformin reduces glucose production, induces fatty acid oxidation and suppresses expression of lipogenic enzymes. Metformin increases insulin-mediated glucose uptake in skeletal muscle. Most of these effects are mediated through activation and phosphorylation of AMPK [17]. AMPK has been found to be expressed in both clonal β-cell lines [18] and intact islet cells [19], and is hypothesized to play a major role in glucose sensing and insulin secretion (reviewed in [20]). Metformin working through AMPK could, therefore, have an effect on insulin secretion in Type 1 diabetes, in patients with residual β-cell function. It could also work via improvements in insulin sensitivity, which resulted in a reduced insulin requirement in early Type 1 diabetes, in two small studies [21,22].

Vella *et al.* published a meta-analysis on metformin use in Type 1 diabetes, summarizing data from five out of nine trials which satisfied their criteria of randomized trials with either a crossover design or where metformin was compared with placebo or a comparator drug [23]. The standardized mean difference (SMD) of HbA1C level between metformin-treated and metformin-free group was -0.10 (95% CI: -0.36–0.15; p = not significant). It has been suggested that in most trials the participants self-titrated insulin to achieve their usual mean glucose explaining similar HbA1C within the groups. Despite the comparable HbA1C, there was a significant reduction in daily insulin dose in the metformin-treated group, with SMD between treatment groups of -0.65 (95% CI: -0.92 to -0.39 U; p < 0.001). This translated into an absolute difference in daily insulin-dose requirement of 6.6 U/day. In the randomized control trial with largest number of subjects there was also a significant reduction of BMI in patients treated with metformin (-0.56 kg/m^2 in metformin against placebo; 95% CI: -1.06 to -0.05) [24].

A major consideration for all glucose-lowering therapies is their impact on the risk of hypoglycemia. Interestingly, in a small study of 27 adolescents in over 3 months (starting dose 500 mg/day increased to 1000 mg/day of metformin) there was significant increase in minor episodes of hypoglycemia in the metformin-treated group compared with placebo [25]. In a second small study on 24 overweight patients with Type 1 diabetes (BMI >25 kg/m^2, mean 29.5 kg/m^2 in the metformin group) treated with metformin (2000 mg/day) or placebo there was significant increase in self-reported biochemical hypoglycemia in the metformin-treated group [26]. In the meta-analysis by Vella *et al.* [23] apart from these two trials there was no significant difference in rates of hypoglycemia in the other seven studies analyzed.

One of the major aims of glucose-lowering therapy is to reduce the risk of cardiovascular events. Estimates have suggested a tenfold increased risk of cardiovascular disease in people with Type 1 diabetes compared with age-matched people without diabetes [27]. Follow-up of 93% of patients from the DCCT trial in the observational Epidemiology Diabetes Interventions and Complications trial, revealed a 42% (95% CI: 9–63%, p = 0.02) reduced risk of cardiovascular events compared with the conventional treatment group [28]. Based upon reported cardiovascular benefits of metformin in Type 2 diabetes, there has recently been an interest in whether treatment with metformin may have a similar benefit on cardiovascular risk reduction in Type 1 diabetes, independent of its effect on glycemic control. This question will, to some extent, hopefully be answered by the ongoing REMOVAL trial, a Phase III clinical study that aims to investigate the effect of metformin on common carotid intima-media thickness as a surrogate marker for cardiovascular disease [29].

There have been no reports of lactic acidosis in the metformin meta-analysis of subjects with Type 1 diabetes [24] and no reports of increased rates of gastrointestinal adverse effects.

Thiazolidinediones

The thiazolidinediones (including pioglitazone and rosiglitazone) are ligands for the peroxisome proliferator activated-receptor Y, which are nuclear receptors expressed in white and brown adipose tissue. Their downstream effects include adipogenesis by promoting re-esterification of free fatty acid leading to reduced plasma free fatty acid levels, therefore, increasing insulin sensitivity. It has been suggested that they

may also improve glucose stimulated insulin secretion [30].

There are conflicting data regarding a potential effect of thiazolidinediones on autoimmunity and the progression of diabetes in animal models of diabetes. While troglitazone and rosiglitazone were found to prevent autoimmune diabetes in non-obese diabetic (NOD) mice [31], pioglitazone was not found to have any advantage over metformin in development of diabetes, although insulitis scores tended to be higher in the pioglitazone-treated group [32]. In a clinical trial in humans, GAD antibody-positive patients classified as having slowly progressive Type 1 diabetes were randomized to receive metformin up to 750 mg/day or pioglitazone at 45 mg/day [33] for 4 years. At the end of intervention, however, a significantly larger proportion of patients in the pioglitazone group were found to become insulin dependent.

In 15 patients with newly diagnosed Type 1 diabetes (mean age: 11.7 years) randomized to receive either pioglitazone at 30 to 45 mg/day or placebo for 24 weeks [34], peak C peptide levels following a mixed meal test was similar between the groups at baseline and there was no significant difference in peak levels between pioglitazone treatment and placebo groups. The authors concluded, therefore, that pioglitazone did not preserve β-cell function.

Finally in a larger study of 60 patients with Type 1 diabetes, randomly assigned to receive placebo or pioglitazone, 30 mg/day for 6 months [35], a decrease in mean HbA1C with pioglitazone was significantly greater than with placebo (-0.22 + 0.29 and -0.06 + 0.49; p = 0.03). More patients achieved HbA1C <7% with pioglitazone compared with placebo, although the change was not significant in either group. There was no significant difference in change of postprandial blood glucose, fasting blood glucose, insulin requirement and BMI. In addition, there was no significant difference in the rate of hypoglycemia between pioglitazone and placebo groups, and there was no report of fluid retention in any participant.

On balance, therefore, while the thiazolidinediones may exert a small benefit on glycemic control, in some subjects, there is little evidence in humans for a benefit on the consequences of autoimmunity.

α-glucosidase inhibitors

α-glucosidase inhibitors inhibit absorption of carbohydrates from the gut by competitive and reversible inhibition of α-glucosidase enzyme, which breaks down carbohydrates into glucose [36]. As this effect is independent of the pancreatic islet, there is reason to believe that they could have an effect in people with Type 1 diabetes.

In a small study, eleven patients with Type 1 diabetes undergoing intensive insulin treatment were treated with miglitol, 50 mg three-times daily [37]. At the end of the treatment period there was significant improvement in mean HbA1C (7.4% pre-treatment to 6.9% at 12 weeks; p = 0.009), BMI (from 20.8 kg/m^2 pre-treatment to 20.4 kg/m^2 at 12 weeks; p = 0.005) and total daily insulin dose (from 36.2 units pretreatment to 33.8 units at week 12; p = 0.012). The patients went on to have a meal tolerance test before and after treatment. A significant improvement in 60 and 120 min glucose at 12 week was also accompanied by an increase in active GLP-1 at 120 min leading the authors to speculate that this was indirect evidence of enhanced endogenous incretin hormone levels.

Although this one small study suggests a glycemic benefit of α-glucosidase inhibition in Type 1 diabetes, further studies are required to confirm these findings.

Amylin analogs

Amylin or islet amyloid polypeptide is a 37 amino acid peptide that is cosecreted with insulin in response to nutrient stimulation and binds to several receptors with high affinity [38]. Endogenous amylin is predisposed to form amyloid deposits, which is hypothesized to play a role in β-cell destruction in Type 2 diabetes [39,40]. Pramlintide is a synthetic peptide that shares most of the pharmacokinetic and pharmacodynamic properties of amylin, without the tendency to accumulate. Amylin has been found to restore glycogen content in liver [41], inhibit glucagon in rats [42] and has binding sites on rodent β-cells [43] with possible effect on insulin secretion. It also slows gastric emptying [44].

Pramlintide infusion has been shown to reduce post-meal glycemia in patients with Type 1 diabetes [45]. Although pramlintide infusion has been reported to have no effect on peripheral insulin sensitivity during euglycemia it has been associated with a significantly higher counter-regulatory hormone response during hypoglycemia [46]. This did not appear, however, to have a clinical impact with respect to recovery from hypoglycemia.

A role for a reduction in postprandial glucose is further supported by a study in which pramlintide was added to usual insulin treatment for 14 days. This resulted in blunting of glucose levels after a standard meal compared with placebo [47]. In the same study there was however significant nausea in the group treated with pramlintide (300 µg before meals). In a further study, patients were randomized to either placebo or three doses of pramlintide with their usual insulin for 2 weeks [48]. At the end of treatment there was significant reduction in AUC of glucose following a standard meal with the higher dose of pramlintide (30 µg four-times a day). The study was too short to report changes in HbA1C, but mean 24 h glucose level post-treatment was significantly reduced in the intermediate dose group only. Other studies have reported improvement in fructosamine levels with pramlintide [49,50]. In a more recent study Type 1 diabetes patients on insulin pump treatment were assessed with continuous glucose monitoring after 4 weeks of treatment with pramlintide with usual insulin [51]. Following treatment, subjects had significantly more readings in the euglycemic range with a concomitant reduction in prandial insulin dose. They also reported significant reduction in postprandial glucose and glucagon excursion.

In a 52-week double-blind, placebo control trial by Whitehouse *et al.* patients were randomized to either placebo or pramlintide [52]. There was a significant reduction in HbA1C (0.67% with pramlintide, 0.16% with placebo; $p < 0.0001$). Increases in daily insulin doses were significantly less in the pramlintide group compared with placebo. Also patients on pramlintide were reported to lose weight, while patients on placebo gained weight. In an open-label extension to the study, patients originally randomized to pramlintide were reported to have regained weight after 65 weeks.

The short-term studies reported higher incidence of gastrointestinal symptoms in the pramlintide-treated groups. Rates of hypoglycemia were generally high in the study participants and not significantly different between groups [48,49]. A high withdrawal rate for side effects, including nausea, vomiting and sinusitis, with pramlintide is well reported [52,53]. Although generally gastrointestinal side effects seem to settle within a few weeks. In the longer trials there appear to be higher rates of patient withdrawal due to hypoglycemia [52].

Pramlintide is licensed for use with insulin in patients with Type 1 diabetes in the USA. It remains, however, a subcutaneous injection that needs to be administered at every meal, with little additional apparent benefit for people already on multiple insulin injections.

Incretin-based treatment

Incretin hormones including GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) are secreted from enteral cells in response to nutrients and influence glucose-mediated insulin, glucagon and somatostatin secretion. They also have a role in glucose sensing and disposal. Biological action of endogenous GLP-1 also extends to the brain, heart, liver, blood vessels, and the immune system and inflammation (reviewed in detail in [54]). Endogenous incretin hormones have a half-life of 2–3 min being cleaved by a ubiquitous protease enzyme, DPP-4 [55].

Incretin receptor signalling is potentiated by either using injectable receptor agonists, which are structurally homologous to human GLP-1, or by using oral inhibitors of DPP-4, which increase levels of endogenous incretin hormones. Although there have been reports of reduced GLP-1 secretion in some people with Type 2 diabetes, it is now generally considered that GLP-1 activity is largely maintained [56]. The use of GLP-1 receptor agonists does not attempt to replace an incretin deficiency but via supra-physiological doses, regulate insulin and glucagon secretion, in a way that lowers blood glucose in a glucose-dependent manner. GLP-1 agonists and DPP-4 inhibitors are routinely used in patients with Type 2 diabetes [57]. GLP-1 agonists and DPP-4 inhibitors are routinely used in patients with Type 2 diabetes [58] and evidence from preclinical and some preliminary clinical trials in small numbers of patients suggest that augmenting GLP-1 signaling will have similar effect on insulin and glucagon secretion in patients with Type 1 diabetes, as well.

GLP-1 receptor agonists

The trials examining effect of GLP-1 receptor agonists in rodent models of Type 1 diabetes are summarized in **Table 1**. GLP-1 receptor agonists have been found to improve glucose tolerance and insulin response in NOD mice [59–61]. Although in some animal studies with exendin-4 there was increase in β-cell mass, reduced β-cell apoptosis and increase in insulin +ve cells in

Table 1. Trials on rodent models of Type 1 diabetes with GLP-1 agonists including combination therapy.

Agent tested	Mouse model (age)	β-cell related outcomes	Other outcomes	Ref.
Exendin-4	NOD (4 week)	Increased β-cell mass, insulin content. Improved glucose tolerance. Reduced insulitis scores	Delay in onset of diabetes Increase in $CD4^+$, $CD8^+$ T cells in lymph nodes, reduction in $CD4^+CD25^+Foxp3^+$regulatory T cells in thymus	[58]
Synthetic human GLP1(7–36) amide ± gastrin	NOD (6–8 week)	Combination treatment restored normoglycemia in diabetic mice, increased pancreatic insulin content, β-cell mass, insulin positive cells in duct, reduced β-cell apoptosis	Combination treatment reduced insulin autoantibodies, and combination treated recipient mice protected β-cells from apoptosis in syngenic islet grafts	[59]
Exendin-4 ± anti-CD3 monoclonal antibody	NOD (new onset diabetes)	Exendin-4 treatment improved glucose tolerance and insulin response in anti-CD3 treated group (with exendin-4 there was no difference in rates of β-cell area, proliferation or apoptosis)	Combination treatment group had higher rate of remission compared with either treatment alone: the correction with exendin-4 was most prominent when glucose level was less than 19.4 mM.	[62]
Exendin-4 ± CFA	NOD (new onset diabetes)	Combination treatment resulted in increased insulin content and islet cells stained for insulin	Combination treatment resulted in higher rate of remission in significantly higher percentage of mice, compared with CFA alone.	[61]

+: Coadministration; CFA: Complete Freund's adjuvant; NOD: Non-obese diabetic.

ducts [59–60], this was not confirmed in other studies [61]. When synthetic GLP-1(7–36) amide was used with gastrin there was a reduction of insulin autoantibodies and β-cells in the treated mice appeared to be protected from apoptosis. Co-treatment of exendin-4 with immunotherapy resulted in high rates of remission of diabetes in new onset diabetes in NOD mice [60,62].

The clinical trials using GLP-1 receptor agonists in Type 1 diabetes patients are outlined in **Table 2**. In the early 90s the effect of intravenous infusion of synthetic GLP-1(7–36) amide was tested in patients with Type 1 diabetes [63]. Eight patients with Type 1 diabetes with undetectable C peptide were infused with GLP-1(7–36) amide or saline alongside a closed-loop insulin infusion system to maintain blood glucose levels at 4–5 mM under basal conditions and 6–7 mM for postprandial. GLP-1(7–36) infusion reduced postprandial glycemic excursion and increased glucose utilization with less exogenous insulin being required to maintain target glucose levels, compared to saline control. Other significant changes reported with the GLP-1(7–36) infusion were an increase in free insulin levels with suppression of glucagon and somatostatin release.

In subjects with residual C-peptide levels (post-meal), a GLP-1(7–36) amide infusion to achieve supra-physiologic plasma levels, after a mixed meal resulted in reduced postprandial hyperglycemia but no change in C peptide response [70]. Plasma glucagon and pancreatic polypeptide levels were, however, suppressed with the GLP-1. The effects disappeared as soon as the infusion was stopped and the changes were attributed to the acute effect of GLP-1 on gastric emptying, rather than a direct effect C-peptide release. Further studies support an effect on glucagon suppression rather than a direct effect on the β-cell [64,65]. Following on from these studies with intravenous GLP1, the effects of subcutaneous GLP-1 agonists co-administered with insulin have been evaluated in people with Type 1 diabetes. **Table 2** summarizes the clinical trials in Type 1 diabetes with different preparations of GLP-1 agonists.

In the trials where patients were treated with GLP-1 agonists for short duration (less than 4 weeks), exenatide or liraglutide reduced glucose excursion following a mixed meal [66–69]. A week-long treatment with liraglutide, on patients having continuous glucose monitoring, was found to significantly reduce time spent with high glucose after breakfast and overall glycemic variability [68]. This effect appears to be independent of residual endogenous C peptide production [69].

Some [66,69], but not all [67] studies suggest that improvements in postprandial glucose excursions are associated with an accompanying suppression of postprandial glucagon although this may, in part, be dose dependent [67]. The other

Table 2. Clinical trials using subcutaneous GLP-1 agonists with insulin in Type 1 diabetes patients.

Agent tested	n BMI	C peptide at treatment	HbA1C	Insulin requirement (U/kg/day)	Hypoglycemia and side effects	Other important findings	Ref.
Synthetic human GLP-1 0.63 μg/kg at: 1) 8 h pre- BF, pre-lunch doses 2) 5 days pre-meals	1) 9 BMI 36 ± 5 kg/m^2 2) 8 BMI 34 ± 3 kg/m^2	Meal stimulated CP <100 pM (for all patients)	1) 6.5 ± 0.3% at b (48 mmol/mol) 2) 6.5 ± 0.3% at b (48 mol/mol)	1) 0.79 ± 0.15 at b 2) 0.73 ± 0.07 at b	1) No hypoglycemia 2) No hypoglycemia	1) Reduced pp glucose and glucagon with GLP-1 2) AUC for glucose for 3 h after BF lower with GLP-1	[64]
Exenatide 1.25 +I or 2.5 μg + I or I pre-meal	8 BMI 23.8 ± 2.1 kg/m^2	Minimal/no endogenous CP after meals (for all patients)	7.4 ± 0.7% (57 mmol/mol)	0.9 ± 0.2 at b Significantly reduced when used with exenatide	Hypoglycemia: 1 subject, nausea: 2 subjects	pp AUC of glucose and gastric emptying reduced with both doses versus insulin alone. No changes in glucagon, C peptide	[65]
Liraglutide 1) L titrated to 1.2 mg/d + I for 4 weeks 2) L titrated to 1.2 mg/d +I or I alone for 4 weeks	1) 10: L + I 2) 19 (9: L + I, 10: I) (BMI 18–27 kg/m^2: inclusion criteria)	After iv. glucose + glucagon 1) CP+ ≥60 pM 2) CP- <30 pM	1) 6.6 ± 0.3% (49 mmol/mol) at b to 6.4 ± 0.2% (46 mmol/mol) at 4 weeks 2) with L + I: 7.5 ± 0.2% (58 mmol/mol) at b to 7.0 ± 0.1% (53 mmol/mol) at 4 weeksI: 7.1 ± 0.3% (54 mmol/mol) at b to 6.9 + 0.2% (52 mmol/mol) at 4 weeks (NS)	1) L + I: 0.5 ± 0.06 at b to 0.31 ± 0.08 at 4 weeks. 2) L + I: 0.72 ± 0.08 at b to 0.59 ± 0.06 at 4 weeks.I: 0.62 ± 0.04 at b to 0.64 ± 0.05 at 4 weeks (NS)	CGM data: 1) less time spent with BG <3.9 mM at week 4 vs week 0 2) No difference between week 4 and week 0 in either groupGI side effects in most patients	CP- on L+I: mixed meal + exercise Required less insulin for mixed meal at week 4 vs week 0At pp exercise BG was not different with raised glucagon at week 4 vs week 0	[66]
Liraglutide 0.6 mg/d + I for 1 week.	14 (8 for 28 weeksBMI: 24 ± 2 kg/m^2	Fasting CP < 100 pM (for all patients)	6.6 ± 0.5% (49 mmol/mol) at b In 8 on L for 28 weeks: 6.5% (48 mmol/mol) to 6.1% (43 mmol/mol)	Basal: 24.5 ± 6 to 16.5 ± 6 U/d Bolus: 22.5 ± 4 to 15.5 ± 4 U/d In 8 on L for 28 weeks: Total daily I: 0.65–0.47	CGM data:Time spent in hypoglycemia not different on L vs before L	CGM data: after L:Less time spent at BG >11.1 mM, Less glycemic variability than before	[67]
Exenatide With/without 5 μg 15 mins prior to test (MMTT, IVGTT)	179 CP-: 28.3 ± 1.2. 8 CP+: 26.4 ± 2.2	For CP+: CP >17 pM after MMTT	CP-: 7.5 ± 0.3% (58 mmol/mol), CP+: 7.3 ± 0.3% (56 mmol/mol)	N/A	Gastric emptying delayed with E	33% reduction in glucose excursion after MMTT with E (vs without). CP+: no change in ISR with E. Glucagon: suppressed with E. No difference between CP+ and CP-. No effect of E on IVGTT	[68]
Liraglutide + IFor 180 ± 14 days	27Obese		7.89 ± 0.13% to 7.46 ± 0.13%	Total insulin: 73 ± 6 U/d to 60 ± 4 U/d	No change in frequency of hypoglycemia with L	Bodyweight: 96.20 ± 3.68 kg to 91.56 ± 3.78 kgSystolic BP: 130 ± 3 to 120 ± 4 mm Hg	[69]

AUC: Area under curve; b: Stbaseline; BF: Breakfast; BG: Blood glucose; BP: Blood pressure; CGM: Continuous glucose monitor; CP: C peptide; E: Exenatide; I: Insulin; iv.: Intravenous; IVGTT: Intravenous glucose tolerance test; L: Liraglutide; MMTT: Mixed meal tolerance test; N/A: Not applicable; NS: Not significant; pp: Post-prandial.

factor contributing to suppression of postprandial glycemic excursion with GLP-1 agonists is slowing of gastric emptying [67,69], which appear to be more pronounced with the shorter acting GLP-1 agonists. More recent, longer duration studies also appear to demonstrate a glycemic benefit of GLP-1 agonists in people with Type 1 diabetes [67,71,72]. Improved glycemic control also appears to be associated with reduced insulin requirements. As would be expected, because of the glucose-dependent mode of action, no increases in rates of hypoglycemia have been reported with GLP-1 agonist therapy.

The relative contribution of residual β-cell function has also been evaluated in studies in Type 1 diabetes. In a 4-week trial a significant improvement in HbA1C was observed when liraglutide was coprescribed with insulin in 10 C peptide positive and 9 C peptide negative people with Type 1 diabetes [71]. There was a significant reduction in insulin requirement in the groups treated with liraglutide, and there was a greater reduction in the C peptide-positive subjects. C peptide-positive patients also spent less time in hypoglycemia assessed by continuous glucose monitoring at the end of the treatment period. At the end of the treatment period, less insulin was required at the time of a standard mixed meal and glucagon levels were elevated during exercise. There was no significant difference between glucose levels during exercise before and after treatment. This suggests that the glucagon static effect of liraglutide also appears to be glucose dependent, and does not increase the risk of exercise-induced hypoglycemia when used with insulin. As might be expected, most patients in this trial did report gastrointestinal side effects, including nausea and abdominal distension. Patients on liraglutide also lost weight (mean weight -2.3 + 0.3 kg) compared with insulin (mean weight +0.2 + 0.3 kg). The potentially beneficial effect on weight has been confirmed in a further study of 27 obese Type 1 diabetes patients treated with liraglutide for 180 + 14 days [69].

There is currently a sense of optimism surrounding the potential benefit of GLP-1 agonists as insulin adjuncts in people with Type 1 diabetes, with respect to improvements in blood glucose control, reduced insulin doses, low rates of hypoglycemia and potentially weight loss. Results on ongoing studies are eagerly awaited (NCT01787916 [73] and ISRCTN00290196 [74]).

GLP-1 receptor agonists in Type 1 diabetes post-transplant

Another potential application of GLP-1 treatment in Type 1 diabetics has been to support declining graft function after islet or pancreas allograft. Transplanted islets have been found to retain their ability to respond to GLP-1 [75]. Following islet transplantation although insulin response to meals is partially restored, postprandial hyperglucagonemia seems to persist [76,77]. In a series of prospective but nonrandomized, single-arm studies on a small number of subjects GLP-1 agonist treatment has been found to have some role in reducing insulin requirement [76–80]. In all of the published studies, exenatide is the GLP-1 agonist used. Its effect in suboptimal graft function seems to be short lived and tends to wear off after prolonged treatment. There is uncertainty about whether GLP-1 suppresses postprandial glucagon, but the effects correlate with a number of functioning islets and dose of GLP-1 agonist, making tolerability an important consideration. Further well-designed studies are needed in this area.

DPP 4 inhibitors

The DPP-4 inhibitors are oral tablets that inhibit the DPP-4 enzyme, and increases endogenous GLP-1 levels by delaying their degradation. Their efficacy is modest compared with GLP-1 agonists.

The animal studies using DPP-4 inhibitors are summarized in **Table 3**. DPP-4 inhibitors were found to reverse new-onset diabetes and reduce islet inflammation in streptozocin-treated mice [81]. Long-term treatment with DPP-4 inhibitors improved fasting, postprandial and overall glycemic control in animal models of Type 1 diabetes [81,82]. Human pancreatic tissue from non-diabetic donors has been studied following implantation in NOD mice with severe combined immunodeficiency, and treated with DPP-4 inhibitors and proton pump inhibitors to raise endogenous GLP-1 and gastrin levels [83]. The result was an expansion of functioning β-cell mass in treated mice. The effect of DPP-4 inhibitor treatment was studied in streptozocin-treated mice following islet transplantation [84]. There was no effect on glycemic control, but an improvement in graft survival in the treated group.

The clinical trials with DPP-4 inhibitors in Type 1 diabetes are summarized in **Table 4**. In a prospective crossover trial, effect of 4 weeks

Table 3. Trials using DPP-4 inhibition in animal models of Type 1 diabetes.

Agent tested	Mouse model	β-cell-related outcomes	Other outcomes	Ref.
DPP-4 inhibitor: NVP-DPP728 for 2, 4 and 6 weeks	NOD mice (new onset diabetes)	Increase in pancreatic insulin content, insulin+ and BrdU+ cells in the treated mice	Diabetes was reversed in treated mice: 57% (2 weeks treatment), 74% (4 weeks treatment), 73% (6 weeks treatment) Insulitis was reduced and percentage of $CD4^+CD25^+FoxP3^+$ regulatory T cells increased in treated NOD mice in remission	[82]
DPP-4 inhibitor: DP IV inhibitor (P32/98) for 7 weeks	STZ treated Wistar rats	Improvement in fasting BG, plasma fructosamine levels in treated group OGTT: reduced plasma glucose with increased plasma insulin after oral glucose in treated group. Increased pancreatic insulin content and increased first phase insulin secretion during pancreas perfusion in treated group.	Treated rats gained weight compared with untreated controls	[83]
DPP-4 inhibitor + PPI for 16 weeks after implantation	Human pancreatic cells implanted in NOD-scid mice	IVGTT: Higher human C peptide response and STZ induced hyperglycemia was prevented better in in treated miceInsulin content and insulin+ cells increased in treated mice		[84]
Diet containing Sitagliptin for 5 weeks	STZ-treated mice after mouse islet transplantation	IPGTT: no difference in fasting and post IPGTT glucose levels at end of treatmentIslet graft function and survival was better in sitagliptin-fed mice – as assessed by PET scanning for functional islet mass and glucose stimulated insulin secretion assessed at intermediate time points in both groups.	Plasma glucagon levels were suppressed in Sitagliptin fed group from second to fourth weekSitagliptin-fed mice had lower oral intake but there was no significant difference in weight.	[85]

BG: Blood glucose; BrdU:Bromodeoxyuridine; IPGTT: Intraperitoneal glucose tolerance test; IVGTT: Intravenous glucose tolerance test; NOD: Non-obese diabetic; OGTT: Oral glucose tolerance test; PPI: Proton-pump inhibitor; scid: Severe combined immunodeficiency; STZ: Streptozocin.

of treatment with vildagliptin on postprandial glucose and glucagon was studied in 12 Type 1 diabetics on insulin pump [85]. After a standard breakfast, lower areas under curves for glucose and glucagon were observed. The fasting blood glucose was also lower when on vildagliptin. In a second prospective crossover trial 28 C peptide negative people with Type 1 diabetes were treated with vildagliptin in addition to usual insulin treatment for 4 weeks [86]. Although the insulin requirement did not change, a lower HbA1C was observed after treatment. Subjects had a standard breakfast meal and a hypoglycemic clamp at end of each 4-week treatment period. A suppression of postprandial glucagon was observed with vildagliptin. There was similar increase in levels of counter-regulatory hormones, including glucagon, cortisol, epinephrine, norepinephrine and pancreatic polypeptide during hypoglycemic clamp in both vildagliptin and placebo groups.

In a 4-week placebo controlled crossover trial sitagliptin was not found to have any advantage over placebo in changing insulin requirement or HbA1C [87]. There was also no change in glucose variability with continuous glucose monitoring. In another double-blind, randomized, placebo controlled trial on 141 subjects, 16 weeks treatment with sitagliptin did not have any effect on glucagon response after mixed meal or insulin requirement [88]. The HbA1C, which was significantly lower in the sitagliptin group at the end of a 4-week run-in, remained so at the end-of-treatment period. The HbA1C did not change significantly at the end of 16-week treatment in either the placebo or the sitagliptin group. In the studies with sitagliptin, there was no difference in rates of hypoglycemia or weight versus placebo.

It would appear therefore that on balance, that although some short-term studies have suggested a potential benefit for DPP 4 inhibition as a non-insulin adjunct, this has not been confirmed in longer duration studies.

Leptin

Leptin is an adipokine that has a central and peripheral role in energy homeostasis. Increasing leptin level at the hypothalamus has been found to reduce fasting and postprandial blood glucose level in insulin-deficient rodent models [89,90]. The strategies used for increasing leptin centrally,

without increasing peripheral levels, include intracerebroventricular injections [89] and inducing leptin transgene expression using recombinant adeno-associated virus vector encoding leptin [90]. High central leptin did not affect fasting insulin levels, but was found to significantly reduce postprandial insulin level [89,90]. Insulin sensitivity and glucose disposal was found to be enhanced in the leptin-treated group, suggesting a novel signaling mediated through the hypothalamus, acting through appetite-dependent and -independent signaling [91].

Increasing peripheral levels of leptin has also been investigated. In a study in NOD mice, leptin alone and alongside insulin reduced HbA1C and glycemic variability by suppressing glucagon levels [92]. Low plasma levels of leptin have been reported at diagnosis in Type 1 diabetes, with restoration of physiologic levels within 3–5 days of treatment [93]. Intensive insulin treatment in patients with Type 1 diabetes has been found to be associated with higher plasma level of leptin independent of BMI [94]. It is however unclear whether insulin treatment stimulates leptin secretion [95,96], or indeed if there is a clinical benefit of restoring peripheral leptin levels in the insulin-treated Type 1 diabetic patient. Currently a Phase I trial is underway to test the effect of merteleptin, a synthetic analog of leptin, in Type 1 diabetes [97].

Immunotherapy

In Type 1 diabetes β-cell death is a result of autoreactive T cells and other mononuclear cells infiltrating islets [98]. Autoantibodies, especially insulin specific, appear several years before clinical diagnosis making preventive strategies possible [99,100].

Secondary immune-prevention strategies induce regulatory T cells by exposing mucosa to low doses of insulin. Inhibitory cytokines from Tregs inhibit autoreactive T cells. Trials [101,102,103] so far have shown only modest benefit. Pre-POINT is an on-going Phase I study in children at high genetic risk of Type 1 diabetes, to assess whether mucosal insulin can prevent development of autoantibodies [104]. Vaccination with GAD – alum to induce immune tolerance to GAD-65 – is being tested in clinical trials described in [105].

Nonantigen-specific immunosuppression has been widely tested with only limited success and often results in unsurmountable side effects. While clinical benefit with cyclosporin

Table 4. Clinical trials using DPP-4 inhibitors alongside insulin in Type 1 diabetes.

Agent tested	n BMI	C peptide at treatment	HbA1C	Insulin requirement	Hypoglycemia and side effects	Other important findings	Ref.
Vildagliptin 200 mg/d + I for 4 weeks: crossover with placebo	12 on insulin pumpBMI: 24 ± 2.9 kg/m^2	NA	7.6 ± 0.9% (60 mmol/mol) at baseline	0.49 ± 0.16 U/kg at b	Several hypoglycemic episodes reported	Postbreakfast glucose and glucagon suppressed with V compared with placebo	[86]
Vildagliptin 100 mg/d + I for 4 weeks – crossover with placebo	28BMI: 24.8 ± 3.3 kg/m^2	CP: -ve	V: 7.51 ± 0.11% (58.1 mmol/mol) at b to 7.17 ± 0.10% (54.7 mmol/mol)P: 7.46 ± 0.09% (57.6 mmol/mol) to 7.44 ± 0.09% (57.4 mmol/mol)	Long-acting: 0.37 + 0.07 U/kg, short-acting: 0.37 + 0.09 U/kg at b – not significantly changed by V or P	Mild hypoglycemia: 2 in V and 1 in P and 1 in wash-out), common cold, abdominal pain	Significant suppression of AUC of glucagon after meal with VNo difference in glucagon, C, E, NE and PP during hypoglycemic clamp between V and P	[87]
Sitagliptin 100 mg/d + I for 4 weeks – crossover with placebo	19 S-P:10 P-S: 9 BMI: S-P: 27.6 ± 3.7 P-S: 26.7 ± 2.6	CP: N/A	At b: S-P: 9.5 ± 0.7% (81 mmol/mol), P-S: 9.2 ± 0.7% (77 mmol/mol)In both S-P and P-S: HbA1C declined. Significant decline with S vs P	S vs P: -0.051 + 0.81 U/kg	CGM time spent at <3.1 mM: S vs P: 0.2 ± 0.1	CGM: There was no difference between S and P in glucose variabilityNo difference in weight between S and P	[88]
Sitagliptin 100 mg/d + I for 16 weeks	141	CP+ subgroup	Was significantly lower in S at end of run-in and remained so till end	No change between S and P		No significant difference in AUC for glucagon 4 h after standard meal	[89]

AUC: Area under curve; b:Baseline; C: Cortisol; CP: C peptide; E: Epinephrine; I: Insulin; NA: Not available; NE: Norepinephrine; NS: Not significant; P: Placebo; PP: Pancreatic-polypeptide; S: Sitagliptin; V: Vildagliptin.

A [106,107] and rituximab [108] was only transient, mycophenolate [109] or IL-1 based treatment like canakinumab or anakinra [110] was not useful. Treatment with etanercept resulted in only modest improvement in insulin requirement [111,112]. Co-treatment with antithymocyte globulin and prednisolone had to be terminated prematurely for thrombocytopenia [113]. The AIDA study is currently investigating anti-IL-1 therapy in newly diagnosed Type 1 diabetes [114]. More recently there has been increased interest in autoantigen-specific strategies that spare the rest of the immune system, and are expected to have a better side-effect profile. Examples of such strategies include using Diapep277 [115] and expanding the pool of Foxp3-expressing Treg cells. These are beyond the scope of this review and the interested reader is directed to [116].

Conclusion

Insulin therapy in Type 1 diabetes is essential for the majority of our patients. While some achieve adequate glycemic control, many do not. Although an increasing number of people with suboptimally controlled Type 1 diabetes are referred for insulin pump therapy and even β-cell replacement therapy in form of islet or whole organ pancreas transplantation, there are still large numbers struggling on basal bolus therapy. The recent observations on persistence of insulin microsecretion long after diagnosis, and the appreciation that diabetes is a bihormonal disorder of not just abnormal insulin but also glucagon secretion, has generated renewed interest in the role of non-insulin adjunct therapies. While none seem likely to replace insulin, some of these therapies may have potential benefits when used alongside insulin including, improved glycemic control, less hypoglycemia, a beneficial effect on weight management and hopefully a reduction in long-term complications.

Future perspective

Management of patients with Type 1 diabetes is likely to become increasingly reliant on non-insulin adjunctive therapies in addition to usual insulin. The strategies described in this review focus mainly on the metabolic management to achieve glycemic goals. Glucagon is likely to play a central role in addition to insulin [117], as therapies aimed at reducing fasting and postprandial glucagon level like incretin based therapies are now being routinely tested in patients with Type 1 diabetes. GLP-1-based treatment is likely to become available for patients with Type 1 diabetes in the future. Glucagon itself is being used alongside insulin in bihormonal 'bionic' pumps [118] and may be preferred over the current insulin-only pumps. Strategies to increase insulin sensitivity of peripheral organs such as metformin and leptin are more likely to be of benefit in the insulin-resistant Type 1 patients. Exciting advances are also being made with immunotherapy in prolonging remission in newly diagnosed Type 1 diabetics. Autoantigen-specific therapies have far fewer side effects and are likely to become available for routine use.

Financial & competing interests disclosure

C Ballav has a research fellowship from Novonordisk UK Research foundation. SCL Gough recevies honoraria for lectures given on behalf of Novo Nordisk, Sanofi, Lilly, Boehringer Ingleheim (BI), Takeda, GSK, Pfizer, consultancy fees from Novo Nordisk, Sanofi, Lilly, BI, Takeda, GSK, Pfizer, research/educational grant support from Novo Nordisk, Sanofi, Takeda, BI and sponsored clinical trials with Novo Nordisk, Lilly, Sanofi. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Gordon-Dseagu VLZ, Shelton N, Mindell J. Diabetes, overweight/obesity and all-cause and cause-specific mortality: evidence from the health survey for England and Scottish Health Survey linked to mortality data. *Diabetic Med.* 30, 136–136 (2013).

2 The Diabetes Control and Complications Trial group. Hypoglycemia in the diabetes control and complications trial. *Diabetes* 46(2), 271–286 (1997).

3 Mccrimmon RJ, Sherwin RS. Hypoglycemia in type 1 diabetes. *Diabetes* 59(10), 2333–2339 (2010).

4 The UK Hypoglycaemia Study group. Risk of hypoglycaemia in types 1 and 2 diabetes: effects of treatment modalities and their duration. *Diabetologia* 50(6), 1140–1147 (2007).

• **Key trial on adjunctive treatment.**

5 Wang L, Lovejoy NF, Faustman DL. Persistence of prolonged C-peptide production in type 1 diabetes as measured with an ultrasensitive C-peptide assay. *Diabetes Care* 35(3), 465–470 (2012).

6 Keenan HA, Sun JK, Levine J et al. Residual insulin production and pancreatic beta-Cell turnover after 50 years of diabetes: joslin medalist study. *Diabetes* 59(11), 2846–2853 (2010).

• **Key trial on adjunctive treatment.**

7 Oram RA, Jones AG, Besser RE et al. The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning beta cells. *Diabetologia* 57(1), 187–191 (2014).

8 Meier JJ, Bhushan A, Butler AE, Rizza RA, Butler PC. Sustained beta cell apoptosis in patients with long-standing type 1 diabetes: indirect evidence for islet regeneration? *Diabetologia* 48(11), 2221–2228 (2005).

9 Steffes MW, Sibley S, Jackson M, Thomas W. Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care* 26(3), 832–836 (2003).

10 Yki-Jarvinen H, Koivisto VA. Natural course of insulin resistance in type I diabetes. *N. Engl. J. Med.* 315(4), 224–230 (1986).

11 Gray RS, Cowan P, Duncan LJ, Clarke BF. Reversal of insulin resistance in type 1 diabetes following initiation of insulin treatment. *Diabet. Med.* 3(1), 18–23 (1986).

12 Muller WA, Faloona GR, Unger RH. Hyperglucagonemia in diabetic ketoacidosis. Its prevalence and significance. *Am. J. Med.* 54(1), 52–57 (1973).

13 Gerich JE, Lorenzi M, Bier DM et al. Prevention of human diabetic ketoacidosis by somatostatin. Evidence for an essential role of glucagon. *N. Engl. J. Med.* 292(19), 985–989 (1975).

14 Porksen S, Nielsen LB, Kaas A et al. Meal-stimulated glucagon release is associated with postprandial blood glucose level and does not interfere with glycemic control in children and adolescents with new-onset type 1 diabetes. *J. Clin. Endocrinol. Metab.* 92(8), 2910–2916 (2007).

15 Kawamori D, Kurpad AJ, Hu J et al. Insulin signaling in alpha cells modulates glucagon secretion in vivo. *Cell Metab.* 9(4), 350–361 (2009).

16 Paranjape SA, Chan O, Zhu W et al. Influence of insulin in the ventromedial hypothalamus on pancreatic glucagon secretion in vivo. *Diabetes* 59(6), 1521–1527 (2010).

17 Zhou G, Myers R, Li Y et al. Role of AMP-activated protein kinase in mechanism of metformin action. *J. Clin. Invest.* 108(8), 1167–1174 (2001).

18 Salt IP, Johnson G, Ashcroft SJH, Hardie DG. AMP-activated protein kinase is activated by low glucose in cell lines derived from pancreatic beta cells, and may regulate insulin release. *Biochem J.* 335, 533–539 (1998).

19 Leclerc I, Woldersdorf, W., Diraison, F. And Rutter, G. A.. Glucose regulation of 5 AMP activated protein kinase in intact islets. *Diabetic Med.* 20(S2), A72 (2003).

• **Key trial on adjunctive treatment.**

20 Fu A, Eberhard CE, Screaton RA. Role of AMPK in pancreatic beta cell function. *Mol. Cell. Endocrinol.* 366(2), 127–134 (2013).

21 Gin H, Messerchmitt C, Brottier E, Aubertin J. Metformin improved insulin resistance in type I, insulin-dependent, diabetic patients. *Metabolism* 34(10), 923–925 (1985).

22 Pagano G, Tagliaferro V, Carta Q et al. Metformin reduces insulin requirement in Type 1 (insulin-dependent) diabetes. *Diabetologia* 24(5), 351–354 (1983).

23 Vella S, Buetow L, Royle P, Livingstone S, Colhoun HM, Petrie JR. The use of metformin in type 1 diabetes: a systematic review of efficacy. *Diabetologia* 53(5), 809–820 (2010).

•• **Key review paper of relevance.**

24 Lund SS, Tarnow L, Astrup AS et al. Effect of adjunct metformin treatment in patients with type-1 diabetes and persistent inadequate glycaemic control. A randomized study. *PLoS One* 3(10), e3363 (2008).

25 Laing SP, Swerdlow AJ, Slater SD et al. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. *Diabetologia* 46(6), 760–765 (2003).

26 Nathan DM, Cleary PA, Backlund JY et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N. Engl. J. Med.* 353(25), 2643–2653 (2005).

27 Hamilton J, Cummings E, Zdravkovic V, Finegood D, Daneman D. Metformin as an adjunct therapy in adolescents with type 1 diabetes and insulin resistance: a randomized controlled trial. *Diabetes Care* 26(1), 138–143 (2003).

28 Jacobsen IB, Henriksen JE, Beck-Nielsen H. The effect of metformin in overweight patients with type 1 diabetes and poor metabolic control. *Basic Clin. Pharmacol. Toxicol.* 105(3), 145–149 (2009).

29 REducing With MetfOrmin Vascular Adverse Lesions in Type 1 Diabetes (REMOVAL). www.clinicaltrials.gov/show/NCT01483560

30 Ahmadian M, Suh JM, Hah N et al. PPARgamma signaling and metabolism: the good, the bad and the future. *Nat. Med.* 19(5), 557–566 (2013).

•• **Key review paper of relevance.**

31 Beales PE, Pozzilli P. Thiazolidinediones for the prevention of diabetes in the non-obese diabetic (NOD) mouse: implications for human type 1 diabetes. *Diabetes Metab. Res. Rev.* 18(2), 114–117 (2002).

32 Shigihara T, Okubo Y, Kanazawa Y, Oikawa Y, Shimada A. Thiazolidinediones may not reduce diabetes incidence in type 1 diabetes. *Ann. NY Acad. Sci.* 1079, 365–368 (2006).

33 Shimada A, Shigihara T, Okubo Y, Katsuki T, Yamada Y, Oikawa Y. Pioglitazone may accelerate disease course of slowly progressive type 1 diabetes. *Diabetes Metab. Res. Rev.* 27(8), 951–953 (2011).

34 Tafuri KS, Godil MA, Lane AH, Wilson TA. Effect of pioglitazone on the course of new-onset type 1 diabetes mellitus. *J. Clin. Res. Pediatr. Endocrinol.* 5(4), 236–239 (2013).

35 Bhat R, Bhansali A, Bhadada S, Sialy R. Effect of pioglitazone therapy in lean type 1 diabetes mellitus. *Diabetes Res. Clin. Pract.* 78(3), 349–354 (2007).

• **Key trial on adjunctive treatment.**

36 Bischoff H. The mechanism of alpha-glucosidase inhibition in the management of diabetes. *Clin. Invest. Med.* 18(4), 303–311 (1995).

37 Nagai E, Katsuno T, Miyagawa J et al. Effects of miglitol in combination with intensive insulin therapy on blood glucose control with special reference to incretin responses in type 1 diabetes mellitus. *Endocr. J.* 58(10), 869–877 (2011).

38 Hay DL, Christopoulos G, Christopoulos A, Sexton PM. Amylin receptors: molecular composition and pharmacology. *Biochem. Soc. Trans.* 32(Pt 5), 865–867 (2004).

39 Westermark P, Wernstedt C, Wilander E, Sletten K. A novel peptide in the calcitonin gene related peptide family as an amyloid fibril protein in the endocrine pancreas. *Biochem. Biophys. Res. Commun.* 140(3), 827–831 (1986).

40 Clark A, Wells CA, Buley ID et al. Islet amyloid, increased A-cells, reduced B-cells and exocrine fibrosis: quantitative changes in the pancreas in type 2 diabetes. *Diabetes Res.* 9(4), 151–159 (1988).

41 Young AA, Crocker LB, Wolfe-Lopez D, Cooper GJ. Daily amylin replacement reverses hepatic glycogen depletion in insulin-treated streptozotocin diabetic rats. *FEBS Lett.* 287(1–2), 203–205 (1991).

42 Gedulin BR, Rink TJ, Young AA. Dose-response for glucagonostatic effect of amylin in rats. *Metabolism* 46(1), 67–70 (1997).

43 Wang ZL, Bennet WM, Ghatei MA, Byfield PG, Smith DM, Bloom SR. Influence of islet amyloid polypeptide and the 8–37 fragment

of islet amyloid polypeptide on insulin release from perifused rat islets. *Diabetes* 42(2), 330–335 (1993).

44 Macdonald IA. Amylin and the gastrointestinal tract. *Diabet. Med.* 14 Suppl 2, S24–28 (1997).

45 Kolterman OG, Gottlieb A, Moyses C, Colburn W. Reduction of postprandial hyperglycemia in subjects with IDDM by intravenous infusion of AC137, a human amylin analogue. *Diabetes Care* 18(8), 1179–1182 (1995).

46 Nyholm B, Moller N, Gravholt CH et al. Acute effects of the human amylin analog AC137 on basal and insulin-stimulated euglycemic and hypoglycemic fuel metabolism in patients with insulin-dependent diabetes mellitus. *J. Clin. Endocrinol. Metab.* 81(3), 1083–1089 (1996).

47 Kolterman OG, Schwartz S, Corder C et al. Effect of 14 days' subcutaneous administration of the human amylin analogue, pramlintide (AC137), on an intravenous insulin challenge and response to a standard liquid meal in patients with IDDM. *Diabetologia* 39(4), 492–499 (1996).

48 Thompson RG, Peterson J, Gottlieb A, Mullane J. Effects of pramlintide, an analog of human amylin, on plasma glucose profiles in patients with IDDM: results of a multicenter trial. *Diabetes* 46(4), 632–636 (1997).

• **Key trial on adjunctive treatment.**

49 Thompson RG, Pearson L, Kolterman OG. Effects of 4 weeks' administration of pramlintide, a human amylin analogue, on glycaemia control in patients with IDDM: effects on plasma glucose profiles and serum fructosamine concentrations. *Diabetologia* 40(11), 1278–1285 (1997).

50 Nyholm B, Orskov L, Hove KY et al. The amylin analog pramlintide improves glycemic control and reduces postprandial glucagon concentrations in patients with type 1 diabetes mellitus. *Metabolism* 48(7), 935–941 (1999).

51 Levetan C, Want LL, Weyer C et al. Impact of pramlintide on glucose fluctuations and postprandial glucose, glucagon, and triglyceride excursions among patients with type 1 diabetes intensively treated with insulin pumps. *Diabetes Care* 26(1), 1–8 (2003).

52 Whitehouse F, Kruger DF, Fineman M et al. A randomized study and open-label extension evaluating the long-term efficacy of pramlintide as an adjunct to insulin therapy in type 1 diabetes. *Diabetes Care* 25(4), 724–730 (2002).

53 Edelman S, Garg S, Frias J et al. A double-blind, placebo-controlled trial assessing pramlintide treatment in the setting of intensive insulin therapy in type 1 diabetes. *Diabetes Care* 29(10), 2189–2195 (2006).

• **Key trial on adjunctive treatment.**

54 Drucker DJ. Incretin action in the pancreas: potential promise, possible perils, and pathological pitfalls. *Diabetes* 62(10), 3316–3323 (2013).

•• **Key review paper of relevance.**

55 Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* 214(3), 829–835 (1993).

56 Nauck MA, Vardarli I, Deacon CF, Holst JJ, Meier JJ. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? *Diabetologia* 54(1), 10–18 (2011).

57 Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide-1 [7–36 Amide] but Not of synthetic human gastric-inhibitory polypeptide in patients with type-2 diabetes-mellitus. *J. Clin. Invest.* 91(1), 301–307 (1993).

58 Inzucchi SE, Bergenstal RM, Buse JB et al. Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia* 55(6), 1577–1596 (2012).

59 Hadjiyanni I, Baggio LL, Poussier P, Drucker DJ. Exendin-4 modulates diabetes onset in nonobese diabetic mice. *Endocrinology* 149(3), 1338–1349 (2008).

60 Tian B, Hao J, Zhang Y et al. Upregulating CD4+CD25+FOXP3+ regulatory T cells in pancreatic lymph nodes in diabetic NOD mice by adjuvant immunotherapy. *Transplantation* 87(2), 198–206 (2009).

61 Sherry NA, Chen W, Kushner JA et al. Exendin-4 improves reversal of diabetes in NOD mice treated with anti-CD3 monoclonal antibody by enhancing recovery of beta-cells. *Endocrinology* 148(11), 5136–5144 (2007).

62 Suarez-Pinzon WL, Power RF, Yan Y, Wasserfall C, Atkinson M, Rabinovitch A. Combination therapy with glucagon-like peptide-1 and gastrin restores normoglycemia in diabetic NOD mice. *Diabetes* 57(12), 3281–3288 (2008).

63 Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S. Antidiabetogenic effect of glucagon-like peptide-1 (7–36)amide in normal subjects and patients with diabetes mellitus. *N. Engl. J. Med.* 326(20), 1316–1322 (1992).

64 Creutzfeldt WO, Kleine N, Willms B, Orskov C, Holst JJ, Nauck MA. Glucagonostatic actions and reduction of fasting hyperglycemia by exogenous glucagon-like peptide I(7–36) amide in type I diabetic patients. *Diabetes Care* 19(6), 580–586 (1996).

• **Key trial on adjunctive treatment.**

65 Kielgast U, Asmar M, Madsbad S, Holst JJ. Effect of glucagon-like peptide-1 on alpha- and beta-cell function in C-peptide-negative type 1 diabetic patients. *J. Clin. Endocrinol. Metab.* 95(5), 2492–2496 (2010).

66 Behme MT, Dupre J, Mcdonald TJ. Glucagon-like peptide 1 improved glycemic control in type 1 diabetes. *BMC Endocr. Disord.* 3(1), 3 (2003).

67 Raman VS, Mason KJ, Rodriguez LM et al. The role of adjunctive exenatide therapy in pediatric type 1 diabetes. *Diabetes Care* 33(6), 1294–1296 (2010).

68 Varanasi A, Bellini N, Rawal D et al. Liraglutide as additional treatment for type 1 diabetes. *Eur. J. Endocrinol.* 165(1), 77–84 (2011).

69 Ghazi T, Rink L, Sherr JL, Herold KC. Acute metabolic effects of exenatide in patients with type 1 diabetes with and without residual insulin to oral and intravenous glucose challenges. *Diabetes Care* 37(1), 210–216 (2014).

70 Dupre J, Behme MT, Hramiak IM et al. Glucagon-like peptide I reduces postprandial glycemic excursions in IDDM. *Diabetes* 44(6), 626–630 (1995).

71 Kielgast U, Krarup T, Holst JJ, Madsbad S. Four weeks of treatment with liraglutide reduces insulin dose without loss of glycemic control in type 1 diabetic patients with and without residual beta-Cell function. *Diabetes Care* 34(7), 1463–1468 (2011).

• **Key trial on adjunctive treatment.**

72 Kuhadiya ND, Malik R, Bellini NJ et al. Liraglutide as additional treatment to insulin in obese patients with type 1 diabetes mellitus. *Endocr. Pract.* 19(6), 963–967 (2013).

73 52 Week Trial of Liraglutide in Type 1 Diabetes (LIDO). http://clinicaltrials.gov/ct2/show/NCT01787916

74 ISR CTN. The effect of lixisenatide in type 1 diabetes . www.controlled-trials.com/ISRCTN00290196

75 Fung M, Thompson D, Shapiro RJ et al. Effect of glucagon-like peptide-1 (7–37) on beta-cell function after islet transplantation in type 1 diabetes. *Diabetes Res. Clin. Pract.* 74(2), 189–193 (2006).

76 Peixoto EML, Froud T, Gomes LS et al. Effect of exenatide on gastric emptying and graft survival in islet allograft recipients. *Transplant. Proc.* 43(9), 3231–3234 (2011).

77 Faradji RN, Froud T, Messinger S et al. Long-term metabolic and hormonal effects of exenatide on islet transplant recipients with allograft dysfunction. *Cell Transplant.* 18(10), 1247–1259 (2009).

78 Ghofaili KA, Fung M, Ao Z et al. Effect of exenatide on beta cell function after islet transplantation in type 1 diabetes. *Transplantation* 83(1), 24–28 (2007).

79 Froud T, Faradji RN, Pileggi A et al. The use of exenatide in islet transplant recipients with chronic allograft dysfunction: safety, efficacy, and metabolic effects. *Transplantation* 86(1), 36–45 (2008).

80 Rickels MR, Mueller R, Markmann JF, Naji A. Effect of glucagon-like peptide-1 on beta- and alpha-cell function in isolated islet and whole pancreas transplant recipients. *J. Clin. Endocrinol. Metab.* 94(1), 181–189 (2009).

• **Key trial on adjunctive treatment.**

81 Tian L, Gao J, Hao J et al. Reversal of new-onset diabetes through modulating inflammation and stimulating beta-cell replication in nonobese diabetic mice by a dipeptidyl peptidase IV inhibitor. *Endocrinology* 151(7), 3049–3060 (2010).

82 Pospisilik JA, Martin J, Doty T et al. Dipeptidyl peptidase IV inhibitor treatment stimulates beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes* 52(3), 741–750 (2003).

83 Suarez-Pinzon WL, Rabinovitch A. Combination therapy with a dipeptidyl peptidase-4 inhibitor and a proton pump inhibitor induces beta-cell neogenesis from adult human pancreatic duct cells implanted in immunodeficient mice. *Cell Transplant.* 20(9), 1343–1349 (2011).

84 Kim SJ, Nian C, Doudet DJ, Mcintosh CH. Inhibition of dipeptidyl peptidase IV with sitagliptin (MK0431) prolongs islet graft survival in streptozotocin-induced diabetic mice. *Diabetes* 57(5), 1331–1339 (2008).

85 Foley JE, Ligueros-Saylan M, He YL et al. Effect of vildagliptin on glucagon concentration during meals in patients with type 1 diabetes. *Horm. Metab. Res.* 40(10), 727–730 (2008).

86 Farngren J, Persson M, Schweizer A, Foley JE, Ahren B. Vildagliptin reduces glucagon during hyperglycemia and sustains glucagon counterregulation during hypoglycemia in type 1 diabetes. *J. Clin. Endocrinol. Metab.* 97(10), 3799–3806 (2012).

87 Ellis SL, Moser EG, Snell-Bergeon JK, Rodionova AS, Hazenfield RM, Garg SK. Effect of sitagliptin on glucose control in adult patients with Type 1 diabetes: a pilot, double-blind, randomized, crossover trial. *Diabetic Med.* 28(10), 1176–1181 (2011).

88 Garg SK, Moser EG, Bode BW et al. Effect of sitagliptin on post-prandial glucagon and GLP-1 levels in patients with type 1 diabetes: investigator-initiated, double-blind, randomized, placebo-controlled trial. *Endocr. Pract.* 19(1), 19–28 (2013).

• **Key trial on adjunctive treatment.**

89 Lin CY, Higginbotham DA, Judd RL, White BD. Central leptin increases insulin sensitivity in streptozotocin-induced diabetic rats. *Am. J. Physiol. Endocrinol. Metab.* 282(5), E1084–1091 (2002).

90 Ueno N, Inui A, Kalra PS, Kalra SP. Leptin transgene expression in the hypothalamus enforces euglycemia in diabetic, insulin-deficient nonobese Akita mice and leptin-deficient obese ob/ob mice. *Peptides* 27(9), 2332–2342 (2006).

91 Kalra SP. Pivotal role of leptin-hypothalamus signaling in the etiology of diabetes uncovered by gene therapy: a new therapeutic intervention? *Gene Ther.* 18(4), 319–325 (2011).

92 Wang MY, Chen L, Clark GO et al. Leptin therapy in insulin-deficient type I diabetes. *Proc. Natl Acad. Sci. USA* 107(11), 4813–4819 (2010).

93 Hanaki K, Becker DJ, Arslanian SA. Leptin before and after insulin therapy in children with new-onset type 1 diabetes. *J. Clin. Endocrinol. Metab.* 84(5), 1524–1526 (1999).

• **Key trial on adjunctive treatment.**

94 Azar ST, Zalloua PA, Zantout MS, Shahine CH, Salti I. Leptin levels in patients with type 1 diabetes receiving intensive insulin therapy compared with those in patients receiving conventional insulin therapy. *J. Endocrinol Invest.* 25(8), 724–726 (2002).

95 Utriainen T, Malmstrom R, Makimattila S, Yki-Jarvinen H. Supraphysiological hyperinsulinemia increases plasma leptin concentrations after 4 h in normal subjects. *Diabetes* 45(10), 1364–1366 (1996).

96 Dagogo-Jack S, Fanelli C, Paramore D, Brothers J, Landt M. Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes* 45(5), 695–698 (1996).

97 Chou K, Perry CM. Metreleptin: first global approval. *Drugs* 73(9), 989–997 (2013).

• **Key trial on adjunctive treatment.**

98 Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature* 464(7293), 1293–1300 (2010).

99 Barker JM, Barriga KJ, Yu L et al. Prediction of autoantibody positivity and progression to type 1 diabetes: Diabetes autoimmunity study in the young (DAISY). *J. Clin. Endocrinol. Metab.* 89(8), 3896–3902 (2004).

100 Verge CF, Gianani R, Kawasaki E et al. Prediction of type I diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512bdc/IA-2 autoantibodies. *Diabetes* 45(7), 926–933 (1996).

101 Skyler JS, Krischer JP, Wolfsdorf J et al. Effects of oral insulin in relatives of patients with type 1 diabetes: The Diabetes Prevention Trial--Type 1. *Diabetes Care* 28(5), 1068–1076 (2005).

102 Harrison LC, Honeyman MC, Steele CE et al. Pancreatic beta-cell function and immune responses to insulin after administration of intranasal insulin to humans at risk for type 1 diabetes. *Diabetes Care* 27(10), 2348–2355 (2004).

103 Nanto-Salonen K, Kupila A, Simell S et al. Nasal insulin to prevent type 1 diabetes in children with HLA genotypes and autoantibodies conferring increased risk of disease: a double-blind, randomised controlled trial. *Lancet* 372(9651), 1746–1755 (2008).

104 Diabetes Pre-POINT study. www.diabetes-point.org/nav2uk.html

105 Morales AE, Thrailkill KM. GAD-alum immunotherapy in Type 1 diabetes mellitus. *Immunotherapy* 3(3), 323–332 (2011).

106 Martin S, Schernthaner G, Nerup J et al. Follow-up of cyclosporin A treatment in type 1 (insulin-dependent) diabetes mellitus: lack of long-term effects. *Diabetologia* 34(6), 429–434 (1991).

107 Feutren G, Papoz L, Assan R et al. Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet* 2(8499), 119–124 (1986).

108 Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H et al. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. *N. Engl. J. Med.* 361(22), 2143–2152 (2009).

109 Gottlieb PA, Quinlan S, Krause-Steinrauf H et al. Failure to preserve beta-Cell function with mycophenolate mofetil and daclizumab combined therapy in patients with new-onset type 1 diabetes. *Diabetes Care* 33(4), 826–832 (2010).

110 Moran A, Bundy B, Becker DJ et al. Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. *Lancet* 381(9881), 1905–1915 (2013).

111 Tack CJ, Kleijwegt FS, Van Riel PL, Roep BO. Development of type 1 diabetes in a patient treated with anti-TNF-alpha therapy for active rheumatoid arthritis. *Diabetologia* 52(7), 1442–1444 (2009).

112 Mastrandrea L, Yu J, Behrens T et al. Etanercept treatment in children with new-onset type 1 diabetes: pilot randomized, placebo-controlled, double-blind study. *Diabetes Care* 32(7), 1244–1249 (2009).

113 Eisenbarth GS, Srikanta S, Jackson R et al. Anti-thymocyte globulin and prednisone immunotherapy of recent onset type 1 diabetes mellitus. *Diabetes Res.* 2(6), 271–276 (1985).

114 Mandrup-Poulsen T, Pickersgill L, Donath MY. Blockade of interleukin 1 in type 1 diabetes mellitus. Nature reviews. *Endocrinology* 6(3), 158–166 (2010).

115 Raz I, Ziegler AG, Linn T et al. Treatment of recent-onset type 1 diabetic patients with DiaPep277: results of a double-blind, placebo-controlled, randomized phase 3 trial. *Diabetes Care* 37(5), 1392–1400 (2014).

116 Luo XR, Herold KC, Miller SD. Immunotherapy of type 1 diabetes: where are we and where should we be going? *Immunity* 32(4), 488–499 (2010).

•• **Key review paper of relevance.**

117 Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *J. Clin. Invest.* 122(1), 4–12 (2012).

118 Russell SJ, El-Khatib FH, Sinha M et al. Outpatient glycemic control with a bionic pancreas in type 1 diabetes. *N. Engl. J. Med.* 371(4), 313–325 (2014).