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# Beyond Glucagon-like Peptide-1: Is G-Protein Coupled Receptor Polypharmacology the Path Forward to Treating Metabolic Diseases?

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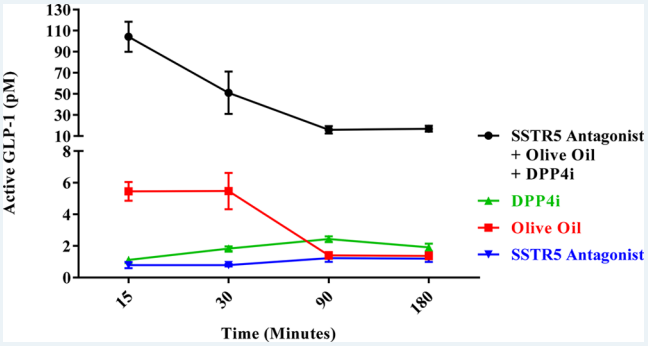
**ABSTRACT:** The glucagon-like peptide-1 receptor (GLP-1R) is a class B G-protein coupled receptor (GPCR) that has proven to be an effective target for developing medicines that treat type 2 diabetes mellitus (T2DM). GLP-1R agonists improve T2DM by enhancing glucose-stimulated insulin secretion, delaying gastric transit, decreasing glucagon levels, and reducing body weight due to anorexigenic actions. The therapeutic successes of these agents helped inspire the design of new multifunctional molecules that are GLP-1R agonists but also activate receptors linked to pathways that enhance insulin sensitization and/or energy expenditure. Herein, these agents are discussed in the context of polypharmacological approaches that may enable even further improvement in treatment outcomes. Moreover, we revisit classical polypharmaceutical GPCR approaches and how they may be utilized for treatment of T2DM. To determine optimal combination regimens, changes in drug discovery practices are likely needed because compensatory mechanisms appear to underlie progression of T2DM and limit the ability of current therapies to induce disease regression or remission.




**KEYWORDS:** *Type 2 diabetes mellitus, exendin-4, G-protein coupled receptor, glucagon, gastric inhibitiory polypeptide, glucagon-like peptide-1, obesity, polypharmacology*

## INTRODUCTION

Type 2 diabetes mellitus (T2DM), obesity, and related comorbidities continue to be unmet medical needs of the 21st century.[1] Fortunately, during the last several years, two new classes of medications have been developed that may lay the groundwork for substantially improving treatment outcomes for patients suffering from these indications: sodium-glucose cotransporter-2 (SGLT2) inhibitors[2] and glucagon-like peptide-1 receptor (GLP-1R) agonists.[3] In addition to robust glucose lowering, a key advance is that drugs within these classes demonstrate cardiovascular (CV) benefits, including reducing the risk of major adverse CV events (MACE) and mortality.[4,5] The efficacy of these agents appears to result from their mechanisms of action. SGLT2 inhibitors work in a noninsulin dependent manner by inhibiting the transporter in the proximal convoluted tubule of the kidney to prevent glucose reabsorption, thus facilitating glucose excretion into urine. GLP-1R agonists activate GLP-1R signaling in pancreatic beta cells and in several other tissues and cell types to improve glycaemic control by enhancing glucose-stimulated insulin secretion, delaying gastric transit, decreasing glucagon, and lowering body weight by an anorexigenic mechanism.[3] The positive CV outcomes data for SGLT2 inhibition and GLP-1R agonism definitively validate these mechanisms for the treatment of metabolic disease. Therefore, it is likely that future drug discovery efforts may focus on these mechanisms. In this regard, the pleiotropic effects that GLP-1R agonists have on multiple regulatory pathways to improve glucose control and total energy balance could provide a foundation for new therapeutic strategies.

The GLP-1R is a class B G-protein coupled receptor (GPCR), and GPCRs overall remain one of the most successful protein families for target-directed drug discovery.[6,7] There are on the order of 300 nonolfactory GPCRs that are potential therapeutic targets. Following the molecular biology revolution and the elucidation of the full complement of the human GPCRome, extensive work in academia and industry has prosecuted GPCR targets to treat several conditions. While agents targeting the amylin receptor (pramlintide as an adjunct to insulin for T1DM or T2DM) and the 5HT2c receptor (lorcaserin for obesity) have been approved by regulatory agencies and launched, the most successful GPCR-based drug discovery campaigns for metabolic disease are the GLP-1R agonists. A variety of factors have contributed to these drugs being the most robust and widely used GPCR ligands for metabolic diseases.[8] This review summarizes key learnings with respect to GLP-1R biology and lays out putative reasons for their efficacy. In our view, the ability of GLP-1R agonists to simultaneously affect independent

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biological pathways leads to the proposal that designed polypharmacology is a mechanism for enhanced benefit. We focus mainly on GPCRs, primarily because the available data best support this target class, and the structural homology of GPCRs provides a tractable opportunity for rational experimentation and discovery.

## THE RATIONALE UNDERLYING COMBINATION THERAPY/POLYPHARMACOLOGY FOR T2DM

Unsurprisingly, polypharmacology/combination therapy is already a strategy utilized for the treatment of several conditions. The use of polypharmacology to treat disease is best exemplified by antipsychotic medicines that target multiple GPCRs and neurotransmitter transporters to induce their therapeutic effect.[9] Combination therapy is also standard practice in the treatment of infectious disease, cancer, hypertension, and T2DM. For instance, the antigluconeogenic drug metformin is widely used in separate or fixed-dose combinations with other medications such as SGLT2 or dipeptidyl peptidase-4 (DPP4) inhibitors. However, in this report, we seek to differentiate between the opportunistic combination of two independently developed drugs versus the *de novo* design of a polypharmacological molecule and fixed-dose combination based on a physiological hypothesis. Much of modern drug discovery is predicated on monogenetic target validation from human genetics and/or genetically engineered rodents, of which unmined opportunities may be low.[8] This is a paradoxical approach for developing agents to treat metabolic disease, as progression to T2DM and obesity for the majority of individuals is generally thought to be driven by environmental factors in concert with a genetic component that is polygenetic in nature.

## BODY WEIGHT REDUCTION FOR T2DM

A better understanding of the peripheral and central mechanisms that regulate energy balance could facilitate the design of potential new T2DM therapies that may offer the metabolic benefits associated with weight loss. The complexity of T2DM suggests that single agents that influence more than one regulatory system or combination therapies affecting complementary pathways are needed to show significant metabolic benefits. Regardless of the approach, new therapies must demonstrate better and more durable efficacy compared with existing medicines in order to improve standard of care. Given this challenge, combined with what we know about progression from prediabetes to frank diabetes, reducing adiposity while also improving glucose control is desirable. Although lowering body weight is difficult, it has been shown that it reduces the incidence of T2DM, and intervention to decrease weight lowers diabetes risk and improves whole body insulin sensitivity.[10−13] Furthermore, in T2DM patients, modest weight loss of 5−10% is associated with at least a 0.5% reduction in HbA1c (several other CV risk factors are also reduced) and even greater benefit occurs with 10−15% weight loss.[14] These findings provide additional rationale for pursuing new therapeutic approaches that target multiple mechanisms, including lowering body weight, for treating T2DM.

## GLP-1R AND THE INCRETIN EFFECT: UNRAVELLING THE GLP-1R MECHANISM

The role of the gut in glucose homeostasis was elucidated by studies demonstrating that insulin secretion in response to oral glucose is substantially larger than in response to intravenously infused glucose.[15] This physiological process is known as the incretin effect, whereby macronutrients such as glucose, lipids, and amino acids stimulate the release of glucoregulatory peptides from cells of the small intestine and colon. The predominant incretins are GLP-1 and glucose dependent insulinotropic polypeptide (GIP).[3] These peptides are subject to nutrient-dependent release from intestinal L- and K-cells into the circulation and act on beta cells of the endocrine pancreas via specific cognate receptors (GLP-1R and GIPR), to enhance insulin secretion and normalize glucose levels. This physiologic circuit is tightly regulated by the rapid inactivation of GLP-1 and GIP by proteolysis.

GLP-1R agonists are primarily thought of as insulinotropic agents via their action on the beta cell. GLP-1R activation causes G$\alpha$s-mediated cAMP production and glucose-dependent insulin secretion in the beta cell, a key antiglycaemic mechanism of GLP-1R agonists. The acute hypoglcycemic actions of GLP-1R are now thought to be just one component of GLP-1 biology. GLP-1R is expressed in extra-pancreatic tissues and GLP-1R agonists have an array of complementary actions that enhance glucose lowering and provide durability of effect.[16] These include rapid physiological effects such as slowing of gastric emptying, suppression of glucagon secretion, and the inhibition of food intake as well as longer-term beneficial effects on the CV system.

Much effort has been undertaken to delineate the contributions of individual cell types and organ systems to the overall antidiabetic pharmacology of GLP-1R agonists. For instance, gastric emptying is a determinant of postprandial hyperglycaemia, and consequently %HbA1c levels;[17] GLP-1R activation slows gastric transit, thus contributing to the overall mechanism whereby GLP-1R agonists improve postprandial hyperglycaemia.[18] Similarly, GLP-1 can independently reduce glucagon secretion, and an important attribute of GLP-1R agonist treatment is the ability to decrease hyperglucagonemia in T2DM patients.[19] Further, in both preclinical models and humans, GLP-1R agonists demonstrate *bona fide* antiobesity effects. Activation of the GLP-1R causes a decrease in energy intake, rather than an increase in energy expenditure, to induce weight loss. Interestingly, recent data indicate that once-weekly GLP-1R agonist therapy with long-acting agents provides better metabolic control than daily GLP-1R agonists that have poorer pharmacokinetic properties,[20,21] suggesting sustained receptor activation is beneficial. There is much interest in understanding the neuronal mechanisms and circuits responsible for the positive effects of GLP-1R activation.

The GLP-1R is expressed throughout both the central (CNS) and the peripheral autonomic (ANS) nervous systems.[22−24] The integration of GLP-1R activation in these areas helps yield the cumulative therapeutic benefit of GLP-1R agonists. Many of the extra-pancreatic effects of GLP-1R agonists are thought to occur by a complicated network of peripheral and CNS/ANS effects.[22,25,26] GLP-1R-mediated actions have been observed in vascular smooth muscle, the gastric antrum and pylorus, enteric neurons, nodose ganglia (vagus), and dorsal root ganglia, as well as several brain regions, including the hypothalamic arcuate and paraventricular nuclei and the area postrema of the brainstem.[23] A critical question that is still being investigated is whether the GLP-1R agonists (large, peptide-based molecules) require direct access to brain regions expressing GLP-1Rs to elicit their metabolic effects. For example, areas outside the blood brain barrier, including the area postrema (a brainstem circumventricular organ) and the nodose ganglion/vagal nerve


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**Table 1. Potential Next Generation GLP1-Based Polyagonists**[a]

| peptide | GLP-1R | GIPR | GCGR | ref and/or ClinicalTrials.gov identifier | phase of clinical development |
|---|---|---|---|---|---|
| TAK-094 | √ | √ | • | 83 | Phase 1 planned |
| NNC0090-2746 | √ | √ | • | 58, NCT02205528 | discontinued |
| RO6807952 | √ | √ | • | NCT01358929 | discontinued |
| LY3298176 | √ | √ | • | NCT02759107 | Phase 2 |
| oxyntomodulin | √ | • | √ | 51 | N/A |
| G49 | √ | • | √ | 84 | N/A |
| MEDI0382 | √ | • | √ | 85, NCT02394314 | Phase 2 |
| SAR425899 | √ | • | √ | NCT02411825 | Phase 2 |
| MK-8521 | √ | • | √ | NCT01982630 | Phase 2, stopped |
| BI 456906 | √ | • | √ | NCT03175211 | Phase 1 |
| LY3305677 | √ | • | √ | NCT02972645 | Phase 1 |
| NNC9204-1177 | √ | • | √ | NCT02941042 | Phase 1 |
| MOD-6031 | √ | • | √ | NCT02692781 | Phase 1, stopped |
| JNJ-64565111 | √ | • | √ | NCT03235219 | Phase 2 |
| OPK-88003 | √ | • | √ | NCT03406377 | Phase 2 |
| NNC9204-0530[b] | • | • | √ | NCT02870231 | Phase 1 |
| HM15211 | √ | √ | √ | NCT03374241 | Phase 1 |
| NNC9204-1706 | √ | √ | √ | NCT03095807 | Phase 1 |

[a]Examples of multireceptor agonists being investigated for the treatment of type 2 diabetes mellitus and/or obesity. For each agent, the pharmacological profile and a representative clinical trial identifier is indicated if possible (clinicaltrials.gov). [b]NNC9204-0530 is being tested in combination with the GLP-1R agonist liraglutide in obese but otherwise healthy patients.

have been implicated in the action of GLP-1.[27,28] In addition, studies have shown that proopiomelanocortin peptide (POMC) and cocaine- and amphetamine-regulated transcript (CART)-expressing neurons of the arcuate nucleus bind and internalize peripherally injected fluorescent liraglutide.[29] POMC neurons have been shown to reside inside the blood brain barrier,[30] suggesting some GLP-1R agonists penetrate into the arcuate nucleus. The presence of fenestrated capillaries and those surrounded by Virchow−Robin spaces may provide the arcuate with privileged access to circulating factors.[31,32] These studies appear to provide the best evidence supporting the hypothesis that direct stimulation of the CNS (POMC/CART neurons) is a significant component of the mechanism whereby GLP-1R agonists induce satiety and ultimately weight loss.[29] However, other recent work shows that deletion of GLP-1R from POMC neurons does not influence the efficacy of exendin-4.[33] Additionally, liraglutide efficacy is unaffected in subjects harboring pathogenic mutations in the melanocortin 4 receptor.[34] These findings suggest that other systems may contribute to GLP-1R-induced weight loss. With GLP-1R agonists now firmly established in the marketplace, efforts to combine this anorectic mechanism with molecules that promote energy expenditure are being investigated with the hope of offering even greater effects on adiposity and glucose tolerance.

Recently, impressive clinical data have expanded the known benefits of GLP-1R agonist treatment beyond glycaemic control and body weight regulation. CV outcome studies for liraglutide demonstrate improvement in overall cardiometabolic health. This includes the prevention of nonfatal myocardial infarction, nonfatal stroke, and CV event induced death.[5] It is not clear whether these improvements are due to specific interactions of GLP-1R agonists with the CV system or indirectly due to effective glycaemic control and weight loss. Overall, the biology of GLP-1R is multifaceted and positive effects of GLP-1R agonists are likely to be the summation of the several physiological processes described above.

## A KEY SUCCESS: THE DISCOVERY AND APPLICATION OF GLP-1R AGONISTS

The discovery and development of GLP-1R targeting drugs was built on basic physiology studies demonstrating that GLP-1R agonists are potent insulinotropic agents with robust glucose lowering efficacy in animal models and humans.[3] Contrary to what is widely posited as "genetic target validation", GLP-1R knockout rodents display only marginally diabetogenic phenotypes. Rather, drug discovery and development was enabled by classical pharmacological experiments with supra-physiological levels of native GLP-1 that elucidated the key physiology and pharmacology of GLP-1R biology. For example, the ability of exogenous GLP-1(7−36) to normalize fasting hyperglycemia in diabetic patients was a key piece of data driving the progression of GLP-1 agonists to the clinic.[35] During the last 15 years, DPP4 resistant, peptide-based GLP-1R agonists have demonstrated strong efficacy in clinical trials, and several have now become entrenched in the marketplace. Much of the development of these agents concentrated on enhancing the poor pharmacokinetics of native GLP-1.

A seminal observation was the discovery of the GLP-1R agonist exendin-4 in the venom of the Gila monster (*Heloderma suspectum*).[36] Endogenous incretin peptides are rapidly inactivated by DPP4 mediated hydrolysis of amino terminal dipeptides. Thus, discovery of the DPP4 resistant peptide exendin-4 (exenatide is the synthetic version) enabled the rapid translation of GLP-1R agonists into drugs.[3] In fact, the publication describing the discovery, purification, and characterization of exendin-4 was in April of 1992[36] and FDA approval of exenatide occurred in April of 2005,[37] an impressively short 13 years from discovery to medicine. Exenatide is dosed as a twice-daily injection.[38] To reduce dosing frequency, coformulation of exenatide with microspheres containing poly(D,L-lactide-*co*-glycolide) enables slow release (once-weekly injection).[39] Lixisenatide was developed by adding six lysines to the C-terminus of exenatide in order to increase the half-life in blood (once-daily injection).[40]



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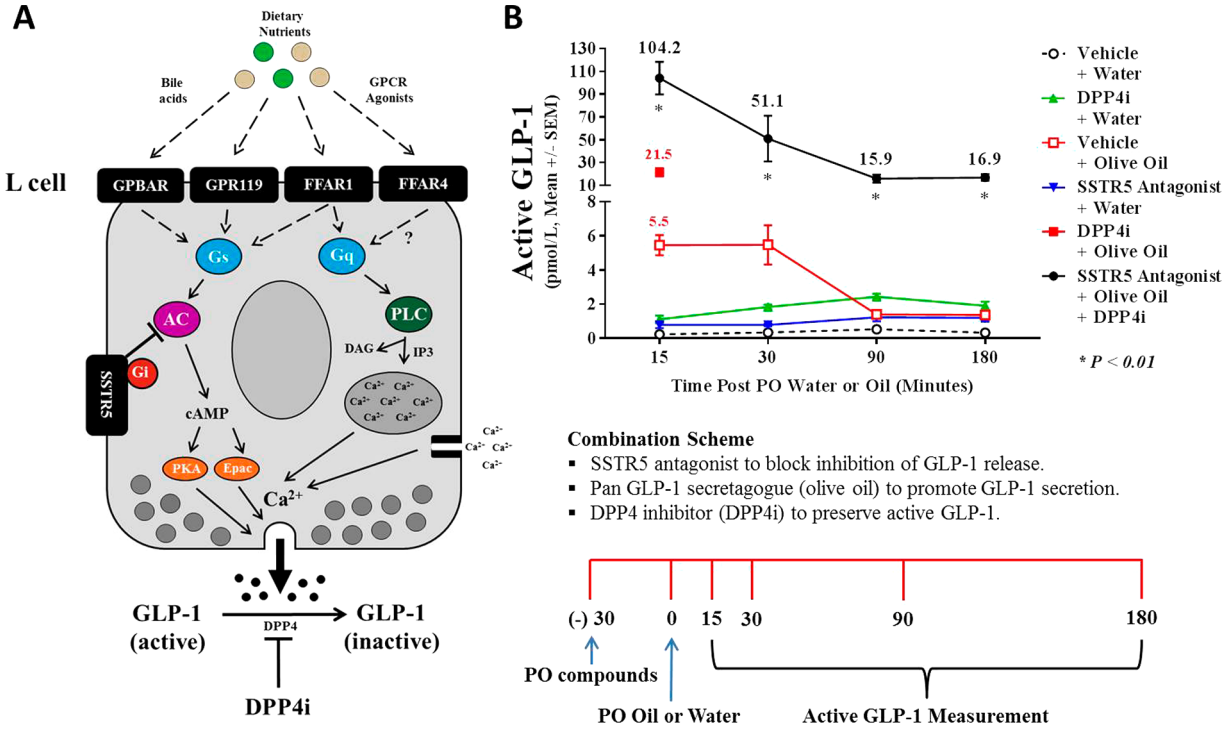


**Figure 1.** Potential combination approaches to increase endogenous concentrations of glucagon-like peptide-1 (GLP-1) for treating metabolic disease. (A) Schematic depiction of putative therapeutic GPCRs expressed in entero-endocrine L cells. (B) The combination of olive oil to provide long-chain fatty acids to promote GLP-1 secretion, a dipeptidyl peptidase-4 (DPP4) inhibitor to preserve active GLP-1, and a somatostatin receptor subtype 5 (SSTR5) antagonist to blunt inhibition of GLP-1 secretion synergistically elevates endogenous GLP-1. Overnight-fasted C57BL/6 mice were given an oral dose of a DPP4 inhibitor (sitagliptin, 10 mg/kg PO) and/or a SSTR5 antagonist (compound 3-1, 30 mg/kg PO), followed 30 min later with an oral bolus of water or olive oil (10 mL/kg PO). Plasma was collected at 15, 30, 90, or 180 min post-water or -olive oil dose for measuring active GLP-1 (Mesoscale Discovery). Data are represented as the mean ± SEM and were compared using one-way ANOVA. The null hypothesis was rejected at $p < 0.05$. Animals were studied and maintained in accordance with the Institutional Animal Care and Use Committee of Eli Lilly and Company, and the Guide for the Use and Care of Laboratory Animals by the National Institutes of Health.

Analogues of GLP-1 include liraglutide (key feature: acylated with palmitic acid attached to Lys26; once-daily injection),[41] dulaglutide (key feature: fused to the Fc component of immunoglobulin G4 heavy chain; once-weekly injection),[42] albiglutide (key feature: fused to human albumin; once-weekly injection),[43] and semaglutide (key feature: acylated with C18 diacid attached via a glutamic acid and double 8-amino-3,6-dioxaoctanoic acid linker; once-weekly injection).[44] All of the approved GLP-1R agonists, described above, have positive effects on glycaemic control in T2DM patients, as assessed by % HbA1c lowering.[45] Moreover, the various molecules demonstrate varied levels of body weight reduction ranging from marginal to clinically significant (e.g., 63% of patients receiving semaglutide 1 mg dose show 5% weight loss).[45,46] GLP-1R agonists are well-tolerated medicines; however, nausea is known to limit dose escalation.[45,47] It is worth noting that DPP4 inhibitors, small molecules that increase circulating GLP-1 and GIP concentrations by inhibition of their degradation, are also effective and safe antidiabetic medications. These molecules are less efficacious than GLP-1R agonists; for example these drugs do not cause weight loss or improve CV outcomes.[48,49]

**Potential Promise of Multifunctional GLP-1R-Based Agonists.** New multifunctional peptide-based therapeutics targeting more than one receptor may provide improved efficacy and tolerability and are currently the focus of considerable research. Given the success of GLP-1R agonists, attention has focused on further enhancing the activity of these therapeutics while mitigating potential issues with tolerability (e.g., nausea) that limit use at higher, more efficacious doses. Oxyntomodulin (OXM) is a 37 amino acid peptide that has the unique biological feature of being an endogenous coagonist activating both the glucagon receptor (GCGR) and the GLP-1R. OXM has comparatively weak *in vitro* affinities for the GCGR and GLP-1R, in relation to its plasma concentration. Thus, it has been suggested that endogenous OXM may not be physiologically important;[50] however, it is clearly of pharmacological relevance given its dual receptor activation capability. Administration of OXM to both rodents and humans[51] has been shown to effectively decrease food intake and body weight. Studies in rodents illustrate that the enhanced body weight lowering activity of OXM is the result of activation of both the GLP-1R and GCGR. The important attribute of this approach is that GCGR activation increases energy expenditure in humans.[52] Therefore, the ability of OXM and OXM-like molecules to reduce caloric intake (through GLP-1R) and promote fuel utilization (through GCGR) offers an attractive therapeutic approach to substantially affect energy balance. Because of this potential synergy, several GCGR/GLP-1R coagonist molecules have been created and advanced to clinical investigation (Table 1). In addition to OXM, GIP is being introduced in new therapeutic approaches. Early work with *Gipr*-deficient mice resulted in confusion on the utility of GIPR modulators.[53] Although GIP was found to enhance insulin secretion from islets, the resistance of *Gipr* null animals to obesity suggested that developing GIPR antagonists would be useful for the treatment of obesity. Work now has largely refocused efforts on


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GIPR agonism, specifically in combination with GLP-1R agonists as a potential new therapeutic strategy. Due to the expression of GIPR in adipocytes, molecules possessing GIPR activity may produce beneficial effects on lipid metabolism that improve insulin resistance.[54] The decline and re-emergence of the GIPR as a target for metabolic disease has been reviewed in extensive detail.[55−57] In turn, it should be pointed out that the role of the GIP/GIPR axis in normal, diabetic, and obese humans is complex, and there remain hypotheses that GIPR antagonism could be viable.[57] Of note, Phase 2 clinical trial data for the GLP-1R:GIPR coagonist NNC0090-2746 (RG7697/RO6811135) was recently published, demonstrating safety and efficacy in a 12-week setting.[58] These findings seem to warrant a definitive test of the coagonist hypothesis in large clinical trials.

Ultimately, the design of new GIPR/GLP-1R coagonists presented an additional complement to the GCGR/GLP-1R coagonists and led to the newest peptides and "unimolecular polypharmacy" as coined by Finan, Tschop, DiMarchi, and colleagues.[59−61] These agents combine the activity and efficacy of simultaneously targeting three GPCRs in the family—GCGR:GIPR:GLP-1R. The success in building molecules with activity at three seemingly distinct GPCRs is made possible by the homology of these receptors and natural ligands, as the GLP-1R is 46% and 49% identical to the GIPR and GCGR, respectively. The discovery and development of incretin ligands with polypharmacology is currently of intense interest. A summary of several publicly disclosed polyagonists is presented in Table 1.

The enhanced efficacy of multitargeted incretins suggests that in the future, it is likely that efforts will extend beyond the class B GPCRs and perhaps toward other peptide receptors. Of potential interest are ligands that modulate the neuropeptide Y (NPY) family of receptors (of which there are five subtypes). NPY administration increases food intake and body weight in rodents. Surprisingly, little effect on food consumption or body weight is observed in NPY knockout mice.[62] Y1, Y5, and Y2 receptor knockout mice exhibit higher body weight and humans expressing an Y2 receptor variant are protected from obesity.[63] Several compounds targeting NPY receptors advanced to clinical studies. The Y5 receptor antagonists MK-0557 and velneperit (S-2367) failed to reduce body weight in obesity trials.[64,65] The Y2 agonist, peptide tyrosine tyrosine (PYY), has demonstrated short-term efficacy in humans,[66] although significant nausea was produced.[67] A mixed Y2/Y4 agonist (TM30338) was advanced into clinical development, but results have not been published. The combination of exenatide and PYY has gained attention due to its synergistic actions on reducing food intake in mice[68] and in obese human subjects.[69] Furthermore, it has recently been disclosed that a new PYY analogue NN9747 is being investigated clinically either alone or in combination with semaglutide.[70] Whether by coformulation or unimolecular coagonists, it will ultimately be important to determine which target(s) or pathway(s) best complement GLP-1R activation to improve insulin sensitization and/or weight loss.

**Can Combinations of Small Molecules Targeting Gut GPCRs Produce Efficacy Similar to the Therapeutic Incretin Peptides?** An alternative to subcutaneously injecting engineered peptides to raise circulating levels of GLP-1 is to increase concentrations of endogenous GLP-1 by orally delivered small molecules that target GPCRs in the gastrointestinal tract. Several GPCRs have been implicated in mechanisms that control GLP-1 secretion (Figure 1A); these include receptors responsive to fatty acids (FFAR1 to FFAR4), lipid derived molecules (GPR119), and bile acids (GPBA). Lipid-activated GPCRs include FFAR1 (GPR40), FFAR2 (GPR43), FFAR3 (GPR41), and FFAR4 (GPR120); for a thorough review, see ref 71. FFAR1 has been heavily studied for T2DM, and agonists are shown to induce insulin secretion from pancreatic beta cells and GLP-1 secretion from intestinal L cells. A relatively new class of FFAR1 agonist/positive allosteric modulators stimulate high levels of GLP-1 secretion.[72] Although preclinical data for several FFAR1 agonists have appeared promising, none have achieved regulatory approval for human use. In addition to fatty acids, GPCRs responsive to lipid-derived molecules have received attention as potential therapeutic targets for T2DM. The best example to date is GPR119 which is activated by oleoylethanolamide and 2-oleoylglycerol.[71] Synthetic agonists to GPR119 induce GLP-1, GIP, and PYY secretion, and lower glucose in preclinical models. Unfortunately, numerous clinical candidates were discontinued, leading to speculation that activation of GPR119 leads to more profound effects in rodents; this may in part be due to the inability of GPR119 agonists to produce robust incretin secretion in humans.[73,74] The other gut GPCR heavily implicated in GLP-1 secretion is GPBA (GPR131 or TGR5). This receptor is activated by bile acids, and in mice, GPBA agonists increase plasma GLP-1 and PYY levels and lower glucose. Unfortunately, activation of this receptor leads to gallbladder distension, an on-target side-effect that does not appear separable from efficacy.[75]

Although a safe and efficacious GLP-1 secretagogue has yet to emerge, recent studies investigating combination approaches may spur further work in this area. For example, combining a DPP4 inhibitor with a FFAR1 agonist increased concentrations of active GLP-1 and synergistically reduced food intake and body weight in diet-induced obese mice.[72] The concept of using combination drug approaches to increase endogenous GLP-1 was further exemplified by also including a somatostatin receptor subtype 5 (SSTR5) antagonist. Here, a regimen consisting of a DPP4 inhibitor, a GLP-1 secretagogue (FFAR1 and/or GPBA agonist), and a SSTR5 antagonist (blunts inhibitory tone on GLP-1 release) resulted in extremely high levels of active GLP-1 that produced profound efficacy in $Lepr^{db/db}$ mice.[76] Similar proof-of-concept findings are shown in Figure 1B where olive oil is used as a general source of long-chain fatty acids to promote GLP-1 secretion; the combination of a DPP4 inhibitor and a SSTR5 antagonist substantially increases active GLP-1 concentrations when mice are administered oral olive oil. For concepts like this to advance, the SSTR5 mechanism needs to be confirmed in humans and a safe GLP-1 secretagogue must become available.

## COULD COMBINATION APPROACHES REVIVE/RESURRECT CNS-TARGETED GPCRS FOR METABOLIC DISEASE?

The CNS is the primary site of action to control food intake for most known pharmacological mechanisms. Unfortunately, targeting receptors in the brain for metabolic disease has fallen out of favor for many pharmaceutical companies due to safety issues observed with several CNS drugs. The most famous, and now infamous, treatment for obesity is the combination of fenfluramine and phentermine (fen-phen). The fen-phen regimen relied on the actions of fenfluramine as a serotonin releasing agent[77] and phentermine as primarily a norepinephrine releaser, although dopamine and serotonin release are also


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reported.[78] Fen-phen produced significant weight loss primarily by its action as an appetite suppressant.[79] However, severe life-threatening side effects (cardiac valvulopathy) caused by ancillary agonism of the 5HT2b receptor[80] required it to be removed from clinical use in 1997. Similarly, rimonabant (cannabinoid receptor 1 (CB1) antagonist) was halted in 2008 due to adverse effects such as depression and suicide, and sibutramine (serotonin-norepinephrine reuptake inhibitor) was withdrawn in 2010 due to concerns of increased CV risk. Therapies available at the time of the writing of this review are phentermine (Lomaira), buproprion-naltrexone combination (Contrave), phentermine-topiramate combination (Qsymia), and liraglutide (Saxenda). These treatments are moderately effective, producing about 5−10% weight loss.[81] Targeting multiple monoamine receptors (via use of reuptake inhibitors such as the serotonin and dopamine reuptake agents or serotonin releasing drugs) or mu opioid receptors in combination with monoamine uptake inhibitors (like the dopamine reuptake inhibitor buproprion) have demonstrated modest efficacy.[81] While safety remains of paramount concern, the ability of small molecules to more readily access receptors in the brain (compared to peptides-based agents) offers the best opportunity of targeting multiple pathways in the CNS that coordinate metabolism. Ultimately, however, efficacy similar to or better than existing therapies, especially GLP-1-based therapies, will be needed.

## A LOOK TO THE FUTURE OF GPCR DRUG DISCOVERY IN METABOLIC DISEASE

Structural homology of the GPCR family provides an opportunity to rationally design agents (small or large molecule) that engage multiple GPCRs to generate synergistic, additive, or complementary benefits through polypharmacology. Similarly, fixed dose combinations are a *bona fide* method of engendering greater efficacy. We propose that rationally designed or empirically discovered GPCR polypharmacology may lead to better outcomes in the design of more efficacious medicines for treating T2DM and obesity.

The increased risk of T2DM due to elevated BMI[82] supports the argument that a successful therapy would impact both body weight and glucose control. Therefore, a critical feature of future efforts should be developing medicines that address the negative effects of excess energy consumption. Ideally, new therapies would (1) improve acute and chronic aspects of glucose metabolism and insulin sensitivity and (2) have an anorexigenic effect to reduce excess calorie intake. Furthermore, because weight loss is accompanied by decreased energy expenditure, a new therapy should also (3) maintain or even increase basal metabolic rate in order to ward off this adaptive response to weight loss. Unfortunately, drug discovery approaches currently used for validating single targets and identifying candidate molecules are not optimally aligned with developing poly-pharmacological therapies. For example, although many medicines are tested in combination paradigms in the clinic, typically these trials are composed of two approved mono-therapies or one approved drug and one investigational molecule. Thus, if synergistic disease-modifying pharmacology could be achieved with two moderately efficacious but unapproved monotherapies in combination, it would likely be difficult to bring to patients under historic drug discovery practices that focused mainly on registration of a single agent.

In summation, we propose that drug discovery resources be applied to efforts aimed at understanding the physiology and genetics of compensatory processes that limit the effectiveness of single agents. An essential advance will be the identification of GPCRs that regulate complementary pathways; these targets will provide substrate for new discovery campaigns. To realize the potential of future polypharmacological GPCR-based medications—either fixed-dose combinations or unimolecular entities—the methods used to discover and develop such drugs will likely need refining.

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### Notes

The authors declare the following competing financial interest(s): All authors are employees of Eli Lilly and Company and may own company stock or possess stock options.

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## ABBREVIATIONS

CART, cocaine- and amphetamine-regulated transcript; CB1, cannabinoid receptor 1; CNS, central nervous system; CV, cardiovascular; DPP4, dipeptidyl peptidase-4; FFAR1, free fatty acid receptor 1; GCGR, glucagon receptor; GIPR, glucose-dependent insulinotropic polypeptide receptor; GLP-1R, glucagon-like peptide-1 receptor; HbA1c, glycated hemoglobin; MACE, major adverse CV events; NPY, neuropeptide Y; OXM, oxyntomodulin; ANS, autonomic nervous system; POMC, proopiomelanocortin peptide; PYY, peptide tyrosine tyrosine; SSTR5, somatostatin receptor subtype 5; T2DM, type 2 diabetes mellitus

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