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Miniseries on promising pharmacological targets for NASH

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Incretins (GLP-1 receptor agonists and dual/triple agonists) and the liver

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Summary

The principle pathological drivers of metabolic dysfunction-associated steatohepatitis (MASH) are obesity and associated insulin resistance, rendering them key therapeutic targets. As glucagon-like peptide 1 receptor agonists (GLP-1RAs) have been licensed for the treatment of diabetes and obesity, they were one of the first drug types to be evaluated in patients with MASH, and successful phase IIa and IIb studies have resulted in progression to phase III clinical trials. Alongside GLP-1RAs, newer combinations with glucagon agonists and/or glucose-dependent insulinotropic peptide (GIP) agonists have been explored in related patient groups, with evidence of improvements in weight, insulin resistance and non-invasive liver parameters. Whether GLP-1RAs have direct, independent effects on MASH or whether they impact on pathophysiology through improvements in weight, insulin resistance and glycaemic control remains a matter of debate. Combinations are being explored, although the potential improvement in efficacy will need to be weighed against the cumulative side-effect burden, potential drug-drug interactions and costs. There is also uncertainty regarding the optimal ratio of glucagon and GIP agonism to GLP-1 agonism in combination agents, and as to whether GIP agonism or antagonism is the optimal approach. Finally, there are also multiple hypothetical permutations combining gut hormone agonists with other emerging assets in the field. Given that the likely dominant mode of action of gut hormone agonists is upstream on weight, initial combinations might focus on agents which have been shown to have a more direct effect on fibrosis, which would include FGF21 and pan-PPAR agonists.

Introduction

Ever since the launch of exenatide in 2005, which ushered in the glucagon-like peptide 1 (GLP-1) agonist era, there has been considerable interest in the use of incretin agonists not only for the control of blood sugar levels in type 2 diabetes, but also to manage other associated conditions including obesity, cardiovascular disease, diabetic kidney disease and, more recently, metabolic dysfunction-associated steatohepatitis (MASH).[1]

With regards to MASH, much initial data focussed on the correlation between GLP-1-induced weight loss and any downstream indirect effects on hepatic steatosis and associated inflammation. With the advent of dual and triple agonists which also target the glucagon receptor and the glucose-dependent insulinotropic peptide (GIP) receptor, there is renewed interest in whether these combination agents can exert synergistic effects and potentially directly affect hepatocyte metabolism. In this review, we examine the evolving evidence on the role of GLP-1 agonists, either alone or as part of combination therapies, in delaying or reversing MASH progression.

Pathophysiology of targets in relation to MASH

Glucagon-like peptide 1

GLP-1 is synthesised in intestinal endocrine cells found predominantly in the distal ileum and colon but also present in the jejunum and duodenum. In the pancreas it stimulates glucose-dependent insulin secretion by beta cells and downregulates secretion of glucagon by alpha cells. These effects in response to GLP-1 agonist therapy are associated with an improvement in glucose control in patients with MASH and impaired glucose tolerance and a reduction in liver fat fraction.

In the brain, GLP-1 acts at the level of the hypothalamus and brainstem to reduce food intake and body weight. Although limited human data are available, GLP-1 receptor expression appears to be decreased in the hypothalamus of patients with type 2 diabetes compared with non-diabetic controls. It is thought that GLP-1 in the central nervous system (CNS) is largely synthesised in the brainstem and transported along axonal networks to the hypothalamus,[2] although recent data demonstrate that liraglutide-induced activation of hypothalamic neurons and its downstream

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Keypoints

- The principle pathological drivers of metabolic dysfunction-associated steatohepatitis (MASH) are obesity and associated insulin resistance, rendering them key therapeutic targets.
- Increased glucose-dependent insulin release and reduced glucagon levels in response to GLP-1 (glucagon-like peptide 1) agonist therapy are associated with a reduction in liver fat fraction, improvements in glucose control and a reduction in liver injury in patients with MASH.
- Glucagon agonist administration drives increased gluconeogenesis and glycogenolysis, reduced hepatic lipid accumulation, increased mitochondrial turnover, improved mitochondrial function, and reduced oxidative stress.
- GIP receptors are expressed within the central nervous system and modulation of these receptors is thought to regulate body weight and food intake, although whether GIP antagonism or agonism is the appropriate way to target these receptors is the subject of debate.
- Combinations of incretins offer the potential for greater weight loss, as well as acting via synergistic mechanisms to enhance beneficial effects on liver injury.
- The efficacy of combination therapy needs to be considered alongside the increased risk of side effects, drug-drug interactions and costs.

metabolic effects are mediated by tanycytic transport into the mediobasal hypothalamus.[3]

Experimental medicine studies in humans show increased brain glucose clearance in response to GLP-1 and that GLP-1 agonists decrease anticipatory food reward.[4,5] These changes are thought to be associated with decreased food intake. Delayed gastric emptying and decreased gastric motility are also seen in response to long-acting GLP-1 agonist therapy although this effect is attenuated over time and is therefore thought to play a minor role with respect to the effects of GLP-1 on weight and glucose control.[6]

GLP-1 activation is also thought to reduce macrophage activity, which may contribute to anti-inflammatory effects in the liver. The therapeutic effect of GLP-1 agonism *vs.* GLP-1/glucagon dual agonism has been compared in C57BL6 mice;[7] mice were treated with the AMYLN MASH-inducing diet for a period of 29 weeks and were then treated with either liraglutide (GLP-1RA), cotadutide (dual GLP-1/glucagon agonist) or obeticholic acid (farnesoid X receptor agonist) for a period of 6 weeks. Liraglutide showed positive effects on serum alanine aminotransferase (ALT) and liver histology (alpha-smooth muscle actin, liver picrosirius red and hydroxyproline) compared to vehicle, alongside a reduction in both hepatic triglyceride and diacylglycerol content. However, the impact on inflammation, fibrosis and the NASH activity score (NAS) *vs.* vehicle was modest. NAS was reduced in more mice treated with cotadutide (83%), compared to OCA (75%), liraglutide (42%), and vehicle controls (8%). Notably, the investigators found no evidence of GLP-1 receptor expression in liver cells, nor in Kuppfer or stellate cells, suggesting that any beneficial effects are mediated extrahepatically. A trial of semaglutide, also in the C57BL6 mouse model,[8] has shown similar results to liraglutide, with a significant improvement in MASH activity and steatosis scores, although there was no impact on lobular inflammation scores.

Any effect of GLP1 agonists on MASH is therefore likely to be indirect and may be related to a reduction in calorie intake, body weight and insulin resistance, all of which lead to reduced liver lipid accumulation and hepatic inflammation (Fig. 1). Data from ob/ob mouse studies suggest that GLP-1 agonists reduce macrophage infiltration into adipose tissue,[9] although whether this effect is also seen in the liver remains unclear and moreover its overall impact remains to be quantified.

Glucagon

Glucagon is primarily produced in pancreatic alpha cells, although some production is also thought to occur within the small intestine. Increased glucagon levels drive increased gluconeogenesis and glycogenolysis and reduce hepatic lipid accumulation. Glucagon also stimulates insulin secretion from beta cells although with a much lower potency compared to GLP-1 and this effect may be due to weak binding of glucagon at the GLP-1 receptor.

With respect to the liver, glucagon has several functions, which include increasing mitochondrial turnover, improving mitochondrial function and reducing oxidative stress, hepatic lipogenesis and steatosis, as well as significantly reducing hepatic glycogen levels and increasing gluconeogenesis. It also reduces stellate cell activation and consequently may impact on fibrosis.

In mice, glucagon has been shown to be thermogenic, increasing energy expenditure via the activation of brown fat.[10] Some human experiments have shown a modest acute effect on energy expenditure,[10] whereas chronic dosing with cotadutide, a GLP-1 and glucagon dual agonist, demonstrated effects on energy intake, potentially via GLP-1 receptors present in the CNS, but no effect on energy expenditure.[11] Thus, although glucagon receptors have been detected in solubilized membranes of human adipose tissue, the cotadutide data raise a question as to whether this finding has any functional significance.[12]

Boland *et al.* explored the effect of 7 days treatment with g1437, a glucagon agonist, in the C57BL6 model – it had profound metabolic effects, with large increases in gluconeogenesis, glycogenolysis and endogenous glucagon production[7] (Fig. 1). Liver lipids were also substantially reduced. Moreover, it appears the effects of glucagon on the liver are at least additive to those of GLP-1; in a separate 29 week study, Boland *et al.* explored the effects of cotadutide *vs.* liraglutide which was dosed to achieve similar weight loss. Their data demonstrated a greater reduction in liver triglycerides,

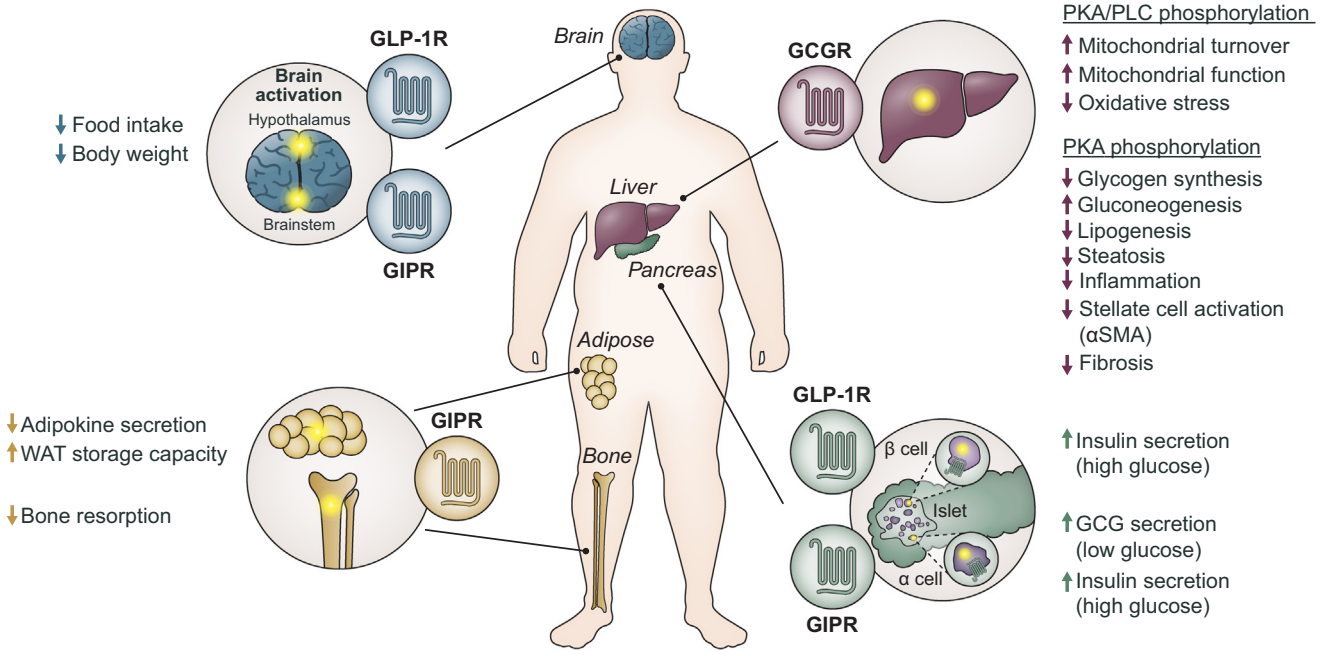

Fig. 1. The major modes and sites of action of the relevant incretin hormones/receptor agonists are detailed. GCG, glucagon; GCGR, glucagon receptor; GIPR, glucose-dependent insulinotropic peptide receptor; GLP-1R, glucagon-like peptide 1 receptor; WAT, white adipose tissue.

diacylglycerol and cholesterol esters with the dual agonist *vs.* the GLP-1 mono-agonist liraglutide. Cotadutide also showed a more significant effect on inflammation and fibrosis *vs.* liraglutide. Kannt *et al.* used the same murine model as Boland, where animals had been treated with a MASH-inducing diet for 36 weeks,[13] and their results showed that a GLP-1/glucagon dual agonist had a greater beneficial effect on markers of MASH disease activity and fibrosis than a weight-matched dose of liraglutide.

Boland *et al.* investigated why glucagon may have additive effects in animals with MASH. They showed that treatment with both g1437 and cotadutide increased mitochondrial turnover and oxidative capacity. This improved metabolic flexibility may therefore underly the benefit of glucagon agonism in MASH, given that the disease is one of chronic energy overload.[7]

The positive effects of glucagon and GLP-1 dual agonism on MASH in the C57BL6 model have now also been confirmed by Nestor *et al.* who carried out an intervention study with pemvidutide, a long-acting dual agonist. After 32 weeks of receiving a MASH-inducing diet, pemvidutide improved steatosis compared to semaglutide and elafibranor, and led to reductions in galectin-3 and collagen type-1 alpha-1.[14]

However, given that glucagon agonism leads to significant hyperglycaemia in the acute phase of dosing, the high prevalence of type 2 diabetes in patients with MASH makes it unlikely that glucagon mono agonists will fit the profile of a safe and effective MASH therapy. Combination with GLP-1 does however appear to blunt glucagon-associated hyperglycaemia without compromising the anti-inflammatory and anti-fibrotic properties of glucagon agonism. In addition, it appears over the longer term that chronic dosing with glucagon may enhance hepatic insulin sensitivity by augmenting insulin activity.[15]

Glucose-dependent insulinotropic peptide

GIP is secreted by K cells of the intestinal epithelium, primarily the proximal duodenum, in response to stimulation by dietary fat. Its main effects are in truncating adipokine secretion and improving white adipose tissue storage capacity, and truncated GIP has been found within pancreatic tissue where it may have a role in alpha/beta cell cross-talk. Indeed, GIP is known to stimulate glucagon secretion even in patients with glucose in the hyperglycaemic range.[16] GIP also appears to act directly on bone by stimulating osteoblast activity. Expression of GIP receptors is noted within the CNS, and receptor modulation is thought to regulate body weight and food intake. Whether GIP antagonism or agonism is the appropriate way to target these receptors is the subject of debate.[17]

The role of GIP agonism as a therapy for MASH is currently unclear. Studies in animal models suggest that GIP enhances lipid deposition in the liver and genetic manipulation to inhibit GIP signalling appears to ameliorate this process (Fig. 1). GIP knockout mice have been shown to have lower levels of hepatic steatosis when fed a high-fat diet and lower levels of IL-6, suggesting that GIP may play a role in mediating inflammatory signalling.[18] There are limited data on the effect of GIP agonists alone in MASH animal models, with data in the C57BL6 model presented at EASD in 2018 by the Hanmi organisation showing that the triagonist for GLP1, glucagon and GIP (HM15211) led to a significantly greater reduction in liver inflammation and steatosis compared to liraglutide alone.[19]

Clinical data and trial results

GLP-1RA

The LEAD studies assessed the role of liraglutide in the management of type 2 diabetes mellitus and obesity. Subsequent

Table 1. Clinical trials with incretin agents.

Drug class	Agent/company	Administration	Phase	Size/duration	Primary end-point	Status/results	
						Inflammation	Fibrosis
GLP-1 agonist	Liraglutide; University of Birmingham & Novo Nordisk	Daily sc	IIa	52 patients; 48 weeks	Histology: resolution of NASH with no worsening of liver fibrosis	NASH resolution with no worsening of fibrosis: 39% in liraglutide group 9% in placebo group $p = 0.019$	Improvement in fibrosis stage with no worsening in NASH: 26% of patients in liraglutide group 14% of patients in placebo group $p = 0.46$
GLP-1 agonist	Semaglutide; Novo Nordisk	Daily sc	IIb	320 patients; 72 weeks	Histology: resolution of NASH with no worsening of liver fibrosis	NASH resolution with no worsening of fibrosis: 40% in 0.1 mg group, 36% in 0.2 mg group, 59% in 0.4 mg group, 17% in placebo group $p < 0.001$	Improvement in fibrosis stage with no worsening in NASH: 43% in 0.4 mg group 33% in placebo group $p = 0.48$
GLP-1 agonist	Semaglutide; Novo Nordisk	Weekly sc	III	1,200 patients; event-dependent	Histology and end-points 1. Sub-part H: resolution of NASH and no worsening of liver fibrosis 2. End-points as per FDA/EMA guidance	Ongoing	Ongoing
GLP-1 & Glucagon dual-agonist	BI 456906; Boehringer Ingelheim	Weekly sc	IIb	1,153 patients; 60 weeks	Histology: improvement of NASH (NAS reduction of 2 or more points)	Recruiting	Recruiting
GLP-1 & Glucagon dual-agonist	ALT-801 Pemvidutide Altimmume	Weekly sc	Ib	72 patients; 12 weeks	Liver fat as measured by MRI-PDFF	Altimmune press release, 68.5% mean reduction in liver fat	Completed, not yet published
GLP-1 agonist & GIP antagonist NCT05669599	AMG 133; Amgen	Monthly sc	IIb	700 patients; 52 weeks	Body weight	Recruiting	Recruiting
GLP-1 & GIP dual-agonist NCT04166773	Tirzepatide; Eli Lilly	Weekly sc	II	196 patients; 52 weeks	Histology: NASH resolution with no worsening of fibrosis	Active - completed recruitment	
GLP-1 & GIP dual-agonist NCT04255433	Tirzepatide; Eli Lilly	Weekly sc	Cardiovascular outcome study	13,299 patients; up to 54 months	Vascular outcomes: 3-part MACE	Active - completed recruitment	
GLP-1 & GIP dual-agonist NCT03882970	Tirzepatide; Eli Lilly	Weekly sc	MRI sub-study	296 patients; 52 weeks	Liver fat as measured by MRI-PDFF	29.78-47.11% reduction in liver fat content across 3 doses	Completed, published

Liraglutide - χ^2 test of the difference between the proportions of patients with histological improvement in each treatment group. Semaglutide - Cochran–Mantel– Haenszel test. MACE, major adverse cardiovascular event; MRI-PDFF, MRI-estimated proton density fat fraction; NAS, NAFLD activity score; NASH, non-alcoholic steatohepatitis.

analyses of these studies demonstrated that treatment with liraglutide led to improvements in serum ALT suggestive of a reduction in liver injury.[20] This led to a proof-of-concept investigator-initiated study, Liraglutide Efficacy and Action in NASH (LEAN) which was a placebo-controlled study in patients with biopsy-proven MASH.[21] The study met its primary endpoint and 39% of patients receiving the 1.8 mg dose of liraglutide achieved histological resolution of MASH with no worsening of liver fibrosis (Table 1). Notably there was greater fibrosis progression in patients receiving placebo (9 *vs.* 36%). The contribution of weight loss to the effectiveness of liraglutide was investigated in the LEAD and LEAN studies – in the former, improvements in ALT were not seen after adjustment for weight loss. In the latter, the odds ratio for an effect on MASH resolution with liraglutide was 4.12 (95% CI 0.66–25.88), although as the confidence interval crossed zero the effect was not significant. In a mechanistic sub-study of LEAN, patients underwent assessments of organ-specific insulin sensitivity, hepatic lipid handling and adipose dysfunction, wherein liraglutide was found to reduce metabolic dysfunction, insulin resistance and lipotoxicity.[22]

Other GLP-1 analogues being investigated include exenatide which was studied in patients with newly diagnosed type 2 diabetes mellitus and metabolic dysfunction-associated steatotic liver disease (MASLD). In this 24-week randomized-controlled multicentre clinical trial 76 patients were randomly assigned 1:1 to receive exenatide or insulin glargine treatment.[23] Both exenatide and insulin glargine reduced liver fat content in patients with drug-naive type 2 diabetes mellitus and MASLD, however, exenatide led to greater reductions in body weight, visceral fat area, liver enzymes, FIB-4 (fibrosis-4) index, postprandial plasma glucose, and LDL-C.

The LEAN study led to a dose-finding phase IIb study, SEMA-NASH, utilising daily subcutaneous semaglutide in a larger 320 patient trial which was 72 weeks in duration.[24] Patients were up-titrated at 4 weekly intervals such that it took 24 weeks to reach the target dose for the highest dose arm. This study also met its primary endpoint of MASH resolution without worsening of liver fibrosis which was achieved in 59% of patients on the 0.4 mg dose of semaglutide. The study did not meet its confirmatory secondary endpoint of fibrosis improvement, perhaps due to the high placebo response rate of 33%. Notably non-invasive markers of liver fibrosis, namely serum enhanced liver fibrosis test and transient elastography improved in patients receiving semaglutide compared with placebo. A pre-specified analysis which looked at fibrosis worsening demonstrated that semaglutide resulted in lower levels of fibrosis progression than placebo, which occured in a dose-dependent fashion. As detailed later in the section on key unanswered questions, it is likely that weight loss with GLP-1 agonists such as semaglutide is an important driver of efficacy in patients with MASH, although they may have additional non-weight-dependent effects, *e.g.* their impact on macrophages. The failure to meet the key secondary endpoint likely reflects the duration of treatment needed and the lack of statistical power for fibrosis in the phase IIb study, although another possibility is the need for adjunctive therapeutic agents. It may also reflect the challenges involved in liver histology, which include sampling issues as well as inter-observer differences in biopsy interpretation. Potential strategies to reduce inter-pathologist variations include artificial intelligence/digital pathology-based approaches, although when applied in the semaglutide studies there was no change in the conclusions regarding liver fibrosis. The incidence of nausea, constipation, and vomiting was higher in the 0.4 mg group than in the placebo group (nausea, 42% *vs.* 11%; constipation, 22% *vs.* 12%; and vomiting, 15% *vs.* 2%) with a discontinuation rate of 7% in the semaglutide arms *vs.* 5% in the placebo arm.

A study of weekly subcutaneous semaglutide in patients with cirrhosis was presented at the International Liver Congress with the intention of demonstrating an improvement in magnetic resonance elastography. It did not meet its primary end-point, nor did it have an effect on liver histology, but it did demonstrate safety in this population as well as metabolic benefits on weight and glycaemia.[25] This lack of improvement in liver fibrosis may reflect the small size of the study, the duration of treatment and/or the refractory nature of fibrosis to GLP-1 treatment.[26] There is now a 1,200 patient phase III clinical trial of semaglutide at a weekly dose of 2 mg, which is expected to report its interim histological end-point in early 2024, pending the later clinical end-point outcomes (NCT04822181).

Switching from subcutaneous to oral semaglutide would make it more acceptable to patients and potentially also reduce associated costs. The coformulation of semaglutide with SNAC (sodium N-[8-(2-hydroxybenzoyl) amino] caprylate) has improved the bioavailability of the oral preparation and reduced the enzymatic digestion of semaglutide as it increases gastric pH. Despite the high pharmacokinetic variability of oral semaglutide, it has been shown to be effective in inducing weight loss and improving glycaemic control in the PIONEER trials,[27] although there have not yet been studies in patients with MASH.

Glucagon

Human mechanistic trial data support a differential effect of glucagon on hepatic metabolism. As expected and demonstrated by Lockton and Poucher, a single glucagon bolus results in a rapid increase in blood glucose due to glycogenolysis but, over the longer term, studies suggest that it is glycogen synthesis that is predominantly affected by glucagon agonists.[28] Parker *et al.* have published data on the effect of cotadutide on hepatic glycogen, showing a 27% reduction after 28 days of treatment along with a 33% reduction in hepatic fat fraction[29] (Table 1). The study also concluded that it was predominantly glycogen synthesis that was affected by chronic glucagon agonism. The same trial also showed a significant reduction in the hepatic concentration of glucogenic amino acids such as alanine and glutamate. Cotadutide is a balanced GLP-1 and glucagon dual agonist with 5-fold higher *in vitro* activity for GLP-1 *vs*. glucagon, and the effects on glycogen were a clear demonstration of glucagon target engagement.[29]

Data from the phase IIb study of cotadutide *vs*. liraglutide or placebo in patients with type 2 diabetes appeared to show differential effects on the liver with respect to both liver fat and liver glycogen[30] with decreases in ALT, aspartate aminotransferase (AST), and gamma-glutamyltransferase levels, as well as improvements in NAFLD fibrosis score and FIB-4 index. PRO-C3 was reduced by approximately 0.5% in individuals treated with 300 ug of cotadutide, whereas it increased by 8% and 13% in those receiving 1.8 mg liraglutide or placebo, respectively. A phase II study of cotadutide at the higher dose of

600 μg daily has now been completed, and although publication of this study is awaited, the redacted clinical study report confirms that significant reductions in hepatic fat fraction, AST and ALT were seen at this dose (NCT04019561). AstraZeneca have now commenced a phase IIb/III study of cotadutide at daily doses of 300 μg and 600 μg in 1,860 participants with non-cirrhotic MASH (NCT05364931).

At the International Liver Congress 2022 meeting Harrison *et al.*[31] presented phase Ib data with pemvidutide, a long-acting GLP1 and glucagon agonist, which led to a greater than 90% reduction in hepatic fat at week 6. The 12-week data were released in a follow-up press release by Altimmune[32] which showed a 68% reduction in hepatic fat fraction at the 1.8 mg dose. It is notable that pemvidutide features an *in vitro* ratio of GLP1:glucagon activity of 1:1, which may drive greater liver target engagement. However, it will be important to evaluate its profile in patients with type 2 diabetes because of the potential of it inducing hyperglycaemia. Notably there was a high dropout rate at the highest dose (24%) due to adverse effects and the published data are awaited.

At EASD 2022, the first data on the effectiveness of BI456906, another glucagon and GLP-1 dual agonist, were presented in patients with type 2 diabetes and obesity by Rosenstock *et al.*[33] A glycated haemoglobin (HbA1c) reduction of 1.88% was reported with weight loss of 6.7% at the highest weekly dose tested (2.7 mg). A relatively high treatment discontinuation rate of 16% was seen in this study, largely related to gastrointestinal adverse events. A trial in MASH is ongoing (NCT04771273).

GIP

Data on the GLP-1 and GIP dual agonist tirzepatide come from the SURMOUNT-1 study which showed a mean percentage change in weight at week 72 of –15.0% with 5 mg weekly dose of tirzepatide, –19.5% with 10 mg dose, and –20.9% with 15 mg dose and –3.1% (95% CI –4.3 to –1.9) with placebo (p <0.001 for all comparisons with placebo).[34] A sub-study of the SURPASS-3 trial was specifically set up to measure liver fat content and all patients had type 2 diabetes, a body mass index above 25 kg/m^2 and a fatty liver index of at least 60.[35] From a baseline liver fat content of 15.7%, the study showed that tirzepatide reduced liver fat by 8.1% over 52 weeks (Table 1), which was associated with a significant reduction in serum ALT, AST and gamma-glutamyltransferase. The change in ALT may indicate a reduction in steatosis or an effect on inflammation and consequent liver injury.

LY3437943 – a novel triple agonist of the glucagon, GIP, and GLP-1 receptors – was shown, in obese mice, to reduce body weight and improve glycaemic control. In a phase I single ascending dose study, retatrutide (LY3437943) showed a safety and tolerability profile similar to other incretins.[36] In a phase II, double-blind, randomized, placebo-controlled trial of adults with a BMI ≥30 or a BMI ≥27 and at least one weight-related condition, the least-squares mean percentage change in weight in the retatrutide groups after 48 weeks treatment was -8.7% in the 1 mg group, -17.1% in the combined 4 mg group, -22.8% in the combined 8 mg group, and -24.2% in the 12 mg group, compared with -2.1% in the placebo group.[37] Similarly, in a phase II trial of patients with type 2 diabetes, HbA1c of 7.0-10.5% (53.0-91.3 mmol/mol), and BMI of 25-50 kg/m^2, -2.02% (0.11 to -22.07 mmol/mol [SD 1.21]) reductions in HbA1c were reported for the 12 mg escalation group by 24 weeks, *vs.* -0.01% (0.21 to -0.12 mmol/mol [SD 2.27]) for the placebo group. By 36 weeks, weight had reduced by 16.94% (SD 1.30) for the 12 mg escalation group, *vs.* 3.00% (0.86) for the placebo group, with a safety profile consistent with GIP and GLP-1 receptor agonists.[38]

Key unanswered biological questions which are pertinent to MASH

There remains a debate as to whether GLP-1RAs have direct, independent effects on MASH or whether they impact on pathophysiology through improvements in weight, insulin resistance and glycaemic control. GLP-1 receptors have been identified on human hepatocytes,[39] and some researchers have postulated that these receptors modulate insulin action in the liver,[39] but more recent data with next-generation antibodies have demonstrated unequivocally that GLP-1 receptors are not expressed in human liver (normal or injured). Similarly, although there are preclinical studies suggesting potential direct effects of GLP-1RAs on *de novo* lipogenesis, lipotoxicity and fatty acid oxidation[22] these are at supra-physiological doses where effects are likely mediated through non-GLP-1 routes. There may be weight-independent effects of GLP-1 such as effects on circulating monocytes which express the GLP-1 receptor, but further data are needed to establish the extent of any weight-independent benefit. Non or inadequate response to GLP-1 agonist therapy remains a significant problem even with potent GLP-1 agonists such as semaglutide. Inadequate responses may be linked to degree of inflammation and fibrosis at baseline, compliance with therapy and lifestyle intervention, or be related to the presence of GLP-1 receptor polymorphisms which impact on the glycaemic and weight response to an intervention.[40]

Given that outcomes in patients with MASH are driven by fibrosis, it is critical that therapies either reverse fibrosis or prevent progression. Whilst GLP-1 analogues have been shown to prevent fibrosis progression, there remains uncertainty as to whether the effects on weight are sufficient to enable fibrosis regression. However, the improvements in non-invasive markers of fibrosis such as serum enhanced liver fibrosis test and transient elastography suggest that histological improvements may be seen in longer, larger studies.

Ratio of receptor activity in incretin combinations and GIP agonism *vs.* antagonism: possible differences in profile between agents

Debate is currently ongoing as to the optimal ratio of glucagon and GIP agonism to GLP-1 agonism in combination therapies, and whether GIP agonism or antagonism is the optimal approach.

The debate about relative potency is particularly important when it comes to GLP-1 and glucagon dual agonists. Cotadutide has an *in vitro* potency for GLP-1 to glucagon activity of 5:1.[41] Clinical trials have shown that it lowers liver fat by around 33% in short-term studies, and has a glucose-lowering effect similar to liraglutide 1.8 mg.[30,42] Survodutide (BI 456906) has similar potency to native GLP-1 *in vitro*, although it is around 6-fold less potent *vs.* endogenous glucagon.[43] A weekly agent, it

has been shown to deliver clinically significant weight loss (20% for the highest two doses) and improved glucose control – a trial on MASH is expected to complete in Q4 2023.[44] In contrast, pemvidutide has an *in vitro* activity ratio of 1:1, which may be reflected in the greater reduction in hepatic fat seen for pemvidutide of up to 90% and in it leading to a potential small increase in blood glucose.[32] Given type 2 diabetes is a significant MASH comorbidity, a more potent liver fat reduction may only be possible in exchange for increased hyperglycaemic risk where GLP-1 and glucagon dual agonists are concerned.

Scientific discussion is ongoing as to whether GIP agonism or antagonism is the desired approach to receptor engagement. Evidence suggests that tirzepatide has a lower affinity for GIP receptors *vs.* native GIP[45] and may therefore operate as a partial GIP antagonist, particularly as – at a high dose – it may drive receptor downregulation over time. Amgen favour a GIP antagonist approach, with AMG-133, a GLP-1 agonist and GIP antagonist currently under development for obesity (NCT04478708).[46] Both receptor affinity and degree of antagonism *vs*. agonism from given agents may play a role in the biological effect, hence the pharmacology of GIP is complex. Long-term outcome data will provide the answer as to which approach is superior.

The optimal ratio of triagonist molecules is yet to be determined, and the first, retatrutide, has now entered clinical development. Data in obese non-diabetic patients suggests weight loss of 22% or more over a 48-week period and Eli Lilly's corporate websites appear to suggest it is associated with a reduction in hepatic fat.[47] The SYNERGY-NASH study with tirzepatide (NCT04166773) is scheduled to read out at the end of 2023 and includes 196 patients with MASH treated with doses of up to 15 mg over 52 weeks. PROXYMO-ADV (NCT05364931) is scheduled to recruit 1,860 patients with MASH randomised to 300 or 600 μg of cotadutide or matched placebo. It is a phase IIb/III study with an initial read out at 48 weeks followed by long-term follow-up for histology at 84 weeks. Until long-term outcomes data in MASH are available it remains to be seen whether the theoretical advantage of cotadutide with respect to glucagon receptor agonism or potentially greater weight loss from GLP-1 and GIP dual agonism translates into a real outcome benefit.

Liabilities and prospects for incretin combinations

All the gut hormone therapies cause nausea and vomiting, and whilst many of these symptoms peak in the first few months of treatment there is a concern about long-term adherence to such drugs. Certainly, the speed at which these agents are up-titrated can have a direct effect on symptoms and adherence, so more gradual up-titration is strongly recommended alongside good access to clinical/nursing teams. In addition, the requirement for subcutaneous administration at this stage may also pose an impediment for some patients, and whilst oral analogues are in development, there remain challenges with drug bio-availability for the oral versions. This alongside longer-term data on the preservation of weight loss will be critical determinants of the therapeutic applicability of these agents. However, despite the aforementioned concerns, these therapies have proven safety profiles and evidence of benefit in reducing long-term cardiovascular outcomes[48,49] which alongside reduction in weight and improvement in glycaemic control make them attractive holistic therapies for patients with MASH.

Combinations with non-incretin assets are being actively considered and there are multiple hypothetical permutations combining gut hormone agonists with emerging assets. Given that the likely dominant mode of action of gut hormone agonists is upstream on weight, initial combinations might focus on agents which have been shown to have a more direct effect on fibrosis, which would include FGF21 (fibroblast growth factor 21) agonists and the pan-PPAR (peroxisome proliferator-activated receptor) agonist lanifibranor.

Whilst the instigation of therapy will be driven by liver disease, MASH fibrosis stage is also an independent predictor of incident cardiovascular disease[50] as well as all-cause mortality.[51] This observational study in 285 patients with MASLD over a follow-up period of 5.2 years showed a marked increase in risk of incident cardiovascular disease (subdistribution hazard ratio 2.86; 95% CI 1.36-6.04) with increasing hepatic fibrosis (F3 or F4). This renders GLP-1 agonists attractive agents as they have a proven beneficial effect on cardiovascular events. However, there is an ongoing debate about the role of GIP in cardiovascular disease. Animal studies are conflicting as to whether GIP agonism is likely to be harmful or protective with respect to cardiovascular events, and human observational data suggests that elevated GIP is associated with increased carotid intima media thickness.[52] The initial tirzepatide phase III programme meta-analysis did not indicate increased cardiovascular risk, although the point estimates for outcome measures had very wide confidence intervals. The hazard ratios comparing tirzepatide *vs*. controls were 0.80 (95% CI 0.57–1.11) for 4-point major adverse cardiovascular events; 0.90 (95% CI 0.50–1.61) for cardiovascular death; and 0.80 (95% CI 0.51–1.25) for all-cause death. SURPASS-CVOT (NCT04255433) will provide a definitive answer as to whether GIP agonism drives a cardiovascular benefit in combination with GLP-1 agonism. This will test the effect of tirzepatide *vs.* dulaglutide on major adverse cardiovascular events in 13,299 patients with type 2 diabetes over a maximum follow-up period of 54 months.

Conclusion

In this review, we discuss whether incretins either alone or in combination are likely to be principal elements of drug therapy in patients with MASH based on their extensive safety profile, pleiotropy of benefits and data on liver histology. The importance of demonstrating the safety of new therapies for MASH was recently reinforced by the FDA. Challenges will be around longer-term compliance and whether the benefits of therapeutic agents are sufficient to impact on liver fibrosis, which is the most important determinant of clinical outcomes.

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Abbreviations

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CNS, central nervous system; GIP, glucose-dependent insulinotropic peptide; GLP-1(RA), glucagon-like peptide 1 (receptor agonist); MASH, metabolic dysfunction-associated steatohepatitis; MASLD, metabolic dysfunction-associated steatotic liver disease; NAS, NAFLD activity score.

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Conflicts of interest

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Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

PNN and PL co-designed and wrote the manuscript.

Supplementary data

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