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Current status of selected oral peptide technologies in advanced preclinical development and in clinical trials

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Abstract

The development of oral dosage forms that allows absorption of therapeutic peptides to the systemic circulation is one of the greatest challenges for the pharmaceutical industry. Currently, a number of technologies including either mixtures of penetration enhancers or protease inhibitors and/or nanotechnology-based products are under clinical development. Typically, these formulations are presented in the form of enteric-coated tablets or capsules. Systems undergoing preclinical investigation include further advances in nanotechnology, including intestinal microneedle patches, as well as their combination with regional delivery to the colon. This review critically examines four selected promising oral peptide technologies at preclinical stage and the twelve that have progressed to clinical trials, as indicated in www.clinicaltrials.gov. We examined these technologies under the criteria of peptide selection, formulation design, system components and excipients, intestinal mechanism of action, efficacy in man, and safety issues. The conclusion is that most of the technologies in clinical trials are incremental rather than paradigm-shifting and that even the more clinically-advanced oral peptide drugs examples of oral bioavailability appear to yield oral bioavailability values of only 1-2% and are, therefore, only currently suitable for a limited range of peptides.

Key words: oral peptides, intestinal permeation enhancers, therapeutic peptides; peptide clinical trials, oral nanotechnology

Contents

1. Introduction

The introduction of human recombinant insulin (Humulin[®], Genentech, USA) in 1981 led to many approved new peptides and proteins in intervening years [1]. Currently, more than 500 peptides are in pre-clinic development, 140 are in clinical trials, and ~60 (i.e. somewhat arbitrarily defined as containing < 50 amino acids) have been approved by the FDA [2]. The rate at which these molecules have reached clinical stages has increased over the last decades: 4.6 in the 1980s, 9.7 in the 1990s, 16.8 in 2000s, and 128 in 2012 [3-6]. Unfavorable physicochemical properties such as large molecular weight (MW), susceptibility to digestive enzymes, hydrophilicity, and low intestinal permeability, mitigate against successful oral peptide delivery, so these molecules are typically administered by injection [7], amounting to a current estimated world-wide annual market of $13 billion [5]. Glatiramer (Copaxone[®], Teva Pharmaceuticals, Israel), leuprolide (Lupron[®], Abbott Laboratories, IL, USA), goserelin (Zoladex[®],

AstraZeneca, UK) and octreotide (Sandostatin®, Novartis, Switzerland) are the four top selling peptides/proteins with projected 2015 sales of > $1 billion each [5].

Among currently marketed peptides, there are oral versions of only eight molecules, split between treatments requiring oral absorption or for retention in the GI tract to treat intestinal disease (Table 1). Developing oral peptide formulations is important because parenteral administration by patients over a chronic period results in poor compliance thereby curtailing efficacy. Despite great improvements in needle technology, it is still estimated that >5% of the population are needle-phobic [8]. Moreover, patients with acromegaly must endure either a painful monthly intra-muscular (i.m.) injections of octreotide or a sub-cutaneous (s.c.) depots of lanreotide, both with low gauge needles, so patient preference for oral is obvious for that example [9]. Secondly, the oral route is a more physiological route of delivery than a bolus injection for certain anti-diabetic peptides (e.g. insulin, MW 5808), where delivery via the portal vein mimics pancreatic release. Commercially, oral delivery also provides an opportunity to extend patent lives with new formulations for expiring injectable peptides. Furthermore, the actual market for the peptide may increase if patients take oral medications earlier in their disease, for example, health outcomes for diabetics appear to improve the earlier in the disease that they transition to insulin [10]. Taking into consideration the enormous challenges to be confronted to achieve systemic peptide absorption following oral administration, the best candidates for oral delivery should be highly potent and have a wide safety margin. In spite of this, insulin remains one of the main candidates for oral delivery [1,4], but it is a particularly problematic molecule due to risks of hypoglycaemia due high inter- and intra-subject variability in oral bioavailability, sensitivity to intestinal proteases, and low permeation across the small intestinal epithelium (Fig. 1). Protection against

degradation can be achieved with adequate formulation, but the low permeability problem is especially difficult to address. Indeed, the large MW of insulin has been considered by some to be close to the maximum for consideration for intestinal permeation in formulations without permeation enhancers [7].

Salmon calcitonin (sCT, MW 3432), is also a well-studied peptide for oral administration. Since it has been marketed in parenteral and nasal forms [11], there is extensive comparator data from these routes of delivery and it is a useful molecule with which to test prototype oral peptide technologies. The FDA and EMA have issued a black box safety warning concerning cancer risk from use of chronic sCT in post-menopausal osteoporotic women, particularly relevant given the relatively narrow risk-to-benefit profile of sCT and the availability of other more efficacious therapeutic options [12,13]. A recent meta-analysis of data for sCT safety with the current marketed formulations however, suggests that there is only a weak association between use of chronic sCT and cancer [14]. Irrespective, an oral recombinant sCT (TBRIA™, Tarsa Therapeutics, Inc. PA, USA) successfully completed Phase III in 2012 for osteoporosis, and a new drug application (NDA) was submitted in 2015 [15]. Other orally-administered peptides are in advanced clinical stages: GLP-1 analogues, parathyroid hormone (PTH) and recombinant (rh)PTH, octreotide (for systemic delivery), and the uroguanylin analogs, plecanatide and dolcanatide (for local delivery) [4,7], and the associated technologies are discussed in more detail here. In the next sections, peptide formulations already marketed, in advanced preclinical study, and those in clinical development are discussed, along with an insight into the potential added value of nanotechnologies compared to conventional technologies.

2. Current oral peptides on the market

2.1 Oral peptides that are systemically absorbed

Cyclosporin A: Sandimmune® was the first oral dosage form of cyclosporin A (MW 1202) (Novartis AG, Switzerland). The combination of the cyclic lipophilic undecapeptide with a non-ionic surfactant containing long chain mono-, di-, and triglycerides (Labrafil® 2125 CS, Gattefossé, France) [17], led to improved overall oral bioavailability of ~25-30%. Nevertheless, there was high intra- and inter-patient pharmacokinetic (PK) variability in a high percentage of patients [18], which was attributed to variation in lipolysis of formulation components, as well as extensive intestinal epithelial P-glycoprotein (P-gp) cyclosporin efflux and cytochrome P450 3A4 (CypP450) intestinal metabolism [19]. In order to overcome the drawbacks of the oil-based Sandimmune®, a self-nanoemulsifying drug delivery systems (SNEDDS) formulation was developed, which forms presented oil droplets smaller than 150 nm after *in vivo* dispersion of the pre-concentrate [20]. This improved formulation, Neoral® (Novartis), promotes better control of droplet size due to the combination of medium chain length mono- and di- triglycerides and medium chain length polyoxyethylene castor oil derivatives with the excipient Cremophor RH40 (BASF, Germany) [21]. Low diameter lipid droplets facilitate solubilisation, resulting in rapid and uniform drug release, while the medium chain fatty acid-based excipients likely increase intestinal permeability directly, and also as a result of inhibiting P-glycoprotein efflux and cytochrome P4503A4 metabolism [22]. Neoral® clinical trials demonstrated enhancement in oral bioavailability, as well as an improved correlation between dose and AUC compared to Sandimmune® (Fig. 2) [20,23–25].

Desmopressin acetate (DDVAP): DDVAP is a hydrophilic potent and stable nonapeptide (MW 1069) first synthesized in solution by Zaoral *et al* [27], while

introduction of solid phase synthesis allowed it to be produced on a commercial scale [28]. Minirin®, Ferring Pharmaceuticals (Denmark) is an analogue of arginine vasopressin with two modifications to boost stability: deamination of the first amino acid, and substitution of the eighth amino acid L-arginine by D-arginine. Recent tablets are designed as fast-melts and retain potency values of 1.6 pg/ml in plasma, but they still yield low oral bioavailability of 0.08% - 0.16% [29,30]. Despite this, it is still effective due to high potency and low cost of synthesis. Desmopressin is an agonist for the vasopressin V2 receptor in kidney tubules and endothelia of blood vessels, thus it is indicated to treat of central diabetes *insipidus*, primary nocturnal enuresis, as well as blood disorders [30–32]. Potential anti-metastatic properties of desmopressin are being investigated in clinical trials [31].

Taltirelin: This thyrotropin-releasing hormone (TRH) analogue is designed to protect the pyroglutamyl peptide bond in TRH from enzymatic hydrolysis [33]. Taltirelin (Ceredist®, Mitsubishi Tanabe Pharma, Japan, MW 477) exhibits higher stability in the blood and brain compared to TRH, which leads to an increase of 10 -100 fold in its CNS stimulatory action compared to TRH, accompanied by a much longer half-life ($t_{1/2}$) [33]. However, taltirelin induces lower thyrotropin (TSH) release in rodents compared to TRH, suggesting a low affinity for receptors in the TSH- secreting cells of the pituitary [34]. Following oral administration to rats and dogs, taltirelin was absorbed through all regions of the small intestine and was detected intact in brain tissue up to 6 h after administration [33]. PK studies in man showed dose-dependent plasma levels, and no evidence of accumulation [33]. Oral taltirelin has been on the Japanese market for fifteen years [35] and oral disintegrating tablets containing taltirelin were recently marketed there as "Ceredist®OD Tablets 5" (Mitsubishi) [35]. There is some evidence for efficacy of this TRH analogue in the treatment of spinocerebellar ataxia and other

neurodegenerative disorders [36,37]. A new TRH analogue with potential for oral delivery is in preclinical development targeting neurodegenerative conditions [38].

Reduced L-glutathione: The natural anti-oxidant, glutathione (GSH**,** MW 307):, is used in as a supplement in the treatment of AIDS-related cachexia, and has been granted GRAS status by the FDA for use as a food additive [39]. Cachexon® (500 mg of encapsulated GSH, Telluride Pharmaceuticals, NJ, USA) was designated as an orphan drug for AIDS-associated cachexia more than two decades ago in the US. However, it appears to have lost its orphan designation and to have been withdrawn [40]. Similarly, an oral glutathione from Mukoviszidose Institut (Bonn, Germany) received orphan designation by the EMA for development for the treatment of cystic fibrosis (CF), which was recently withdrawn [41]. Nevertheless, there is no current oral formulations approved by the FDA [42] solely containing glutathione for the treatment of either cachexia or CF. Capsules containing 500 mg L-glutathione or 300mg L-glutathione plus curcumin, as a possible potentiator [43,44], are sold as natural supplements. Oral L-glutathione (Theranaturals, ID, USA) met primary and secondary endpoints in a placebo-controlled 6-month trial in CF children (NCT02029521), where it significantly improved growth and reduced gut inflammation [45]. In an uncontrolled study, CF patients using oral or inhaled L-glutathione appeared to have improved lung function and increased body weight [46]. L-glutathione from food sources seems to be well absorbed, but intact absorption of the tripeptide from the small intestine *per se* has not been well documented. It is hypothesized that if cleaved, the three amino acids could be used for hepatic glutathione synthesis or excreted in case of excess [47]. Noteworthy is that reduced glutathione appears to have potential as an intestinal permeation enhancer and has been combined to that effect in chitosan-based mucoadhesive formulations in

preclinical studies with poorly permeable molecules [48]. One could argue that reduced GSH is not a pharmaceutical product and is more a nutraceutical, but it was included here as there is clinical trial data.

2.2 Oral peptides that act locally in the intestine

Linaclotide: This anti-secretory molecule has a MW of 1526 Da with three sulfur bridge interactions, creating a rigid tertiary structure which resists intestinal enzymatic degradation of the 14-mer [49,50]. Linaclotide (Linzess®, Ironwood, MA, USA) was approved in 2012 for treatment of irritable bowel syndrome associated with constipation. The mechanism of action is the local activation of guanylyl cylase C (GC-C) following binding to apically-expressed receptors in the small intestine and colon [51], but it is not absorbed. As a result, intracellular enterocyte guanosine monophosphate (cGMP) levels are elevated, causing an increase in electrogenic chloride secretion across human colon [52], accompanied by fluid secretion and resulting in faster GI transit [53]. The oral dosage form consists of microcrystalline cellulose spheres with coatings containing linaclotide with an outer enteric coating, forming beads for loading into a hard gelatin capsule [50]. Research is focused to extend the utility of linaclotide for additional indications and patient populations, as well as investigation on a soluble version of guanylate cyclase (sGC), which may be applied to specific regions of the GI tract [54].

Vancomycin: This antibiotic was first introduced to the market as an injectable antibiotic by Eli Lilly (IN, USA) in the 1950's due to an increase in penicillin-resistance in *staphylococci* [55]. Vancomycin (MW 1449) was never considered a first choice treatment because of the toxicity of early formulations [56]. Intravenous action against

Staphylococcus aureus and *chlostridium difficile* results primarily from inhibition of cell-wall biosynthesis [57]. Vancocin® HCl is not well absorbed orally due to its large MW and hydrophilicity and is only licensed by that route to treat a single colonic condition, pseudomembranous colitis [57], and there have been few efforts to create other oral vancomycin formulations [58]. This is because of the narrow range of conditions that can be treated by local delivery and also because systemic delivery from an oral formulation cannot compete with i.v. to immediately treat life-threatening conditions.

Colistin: This cationic polypeptide is sourced from the antibiotics, the polymycins, or polymycin E. Colistin (MW 1155) is composed of a cyclic heptapeptide and a tripeptide side chain acylated at the N terminus by a fatty acid [59]. It was isolated in 1950 from *Bacillus polymyxa subsp. colistinus* [60], and became commercially available in the 1960s, however due to high systemic toxicity it was replaced [61]. Nowadays, colistin (Koolistin®, Biocon, India) is used as a last-resort drug to treat multi-drug resistant bacterial infections [62]. The main pharmaceutical format commercially available is the prodrug, colistin methanesulfonate (CMS), administered parentally or by inhalation [61]. Oral formulations composed of colistin sulphate are clinically used for local delivery for selective oropharyngeal decontamination (SOD) and selective decontamination of the digestive tract (SDD)[63]. Indication of colistin resistance in *Enterobacteriaceae* after 5 years occurred following oral use for SDD in intensive care units [64]. The EMA recently reviewed the safety and effectiveness of injected or inhaled colistin / CMS, and recommended restricted use [65].

Tyrothricin: This is another antimicrobial cyclic peptide obtained from a natural source, which is commercially available in an oral dosage format. It was first isolated from *Bacillus brevis* [66] and is composed of a mixture of tyrocidins and gramicidins [67]. It has been used for over 60 years to treat infected skin and oro-pharyngeal mucous membranes [67]. In order to overcome systemic toxicity of tyrothricin [68] the peptide is administered locally. Thus, there are several lozenges containing tyrothricin in combination with other compounds (e.g. Tyrozets®, Lemocin®, Dorithricin®, Anginovag®) to treat pharyngitis. Table 1 summarizes the marketed systemically-absorbed and locally-delivered oral peptides.

3. Potential oral candidates not yet on market

The oral peptides in Table 1 typically have MWs of 300-1500 Da. All commercially available peptides above 1000 Da have cyclic structures due either to covalent bonds between amino acids to generate a ring or to disulfide bridges. Cyclic peptides are more resistant to intestinal proteolytic degradation than linear peptides as their tertiary structures are sterically-hindered from binding exo- and endopeptidases active sites [69]. Macrocyclization of peptides is therefore a common strategy to overcome poor intestinal stability of linear peptides (Fig. 3) [70]. They are defined as a ring composed of at least 12 atoms, leading to molecules of MW of 500-2000 Da [71]. Voclosporin (MW 1215, Aurinia Pharmaceuticals, Inc., Canada) is a semi-synthetic *trans* isomer of cyclosporine A, with an additional carbon molecule at the amino acid- 1 residue of cyclosporine A [72]. Oral voclosporin has completed a Phase II trial (NCT00270634 [72]) and is currently in Phase IIb for lupus nephritis (NCT02141672), as well as in a continuing trial for renal transplant patients (NCT01236287). The number of companies focused on macrocycle discovery surged in the last decade and several companies have

reached Phase II with oral peptides [73]. Examples include Ocera Therapeutics Inc. (CA, USA) with TZP-102 (a ghrelin receptor agonist, NCT00889486), Scynexis Inc. (NJ, USA) with SCY-635 (a cyclophilin inhibitor NCT01265511), and Novexel Inc. (France) with NXL-103 (flopristin and linopristin, NCT00949130). It is unclear if any or all of these agents are still in development. Peptidream Inc. (Japan), Ensemble Therapeutics (MA, USA), Stealth Pharma (Boston, NA, USA), Bicycle Therapeutics Limited (UK), and Encycle Therapeutics (Toronto, Canada) are in preclinical development of some macrocycles with potential for oral. Oral bioavailability of macrocycles however, is limited by the large polar surface area that accompanies high amide content [69]. To address this, synthesis of libraries of macrocycles may generate leads with improved oral delivery potential due to high degrees of N-methylation and lower number of hydrogen bond donors [69].

Some pain relieving peptides are potential candidates for oral drug delivery. ImmuPharma PLC (UK) applied its peptide-to-drug converting technology (PDCT) to develop a small peptide, IPP-102199, based on naturally occurring human met-enkephalin [74]. An oral formulation of this peptide is currently in preclinical development. Other interesting molecules are found in venom peptides from spiders, wasps, scorpions and snakes [75]. Apart from roles including pain receptor inhibition, such peptides are being investigated in cancer pathways, neuromuscular diseases, and as antimicrobials. Venom-derived peptides are generally hydrophilic large molecules (~10-40 amino acids), which limit oral bioavailability [75]. On the other hand, structures of venom peptides can be stabilized by disulfide bridges, which increase resistance to luminal proteolysis [76]. RPI-78M is a modified cobra venom toxin polypeptide (8 kDa) [77]. It is under investigation by ReceptoPharm Inc. (FL, USA) as an oral and injectable formulation to treat the orphan disease, juvenile multiple sclerosis [78]. Many other

venom peptides are currently under investigation, however the majority are injectables [76]. An important exception is the Glucagon-like 1 peptide analogue, exenatide, a 4186 Da linear synthetic version of exendin-4 isolated from the venom of the Gila monster lizard, *Heloderma suspectum* [6]. An oral exanatide formulation (ORMD 0901) is in Phase I for Type 2 diabetes (T2D) (Oramed, Jerusalem, Israel). With regard to oral pain relief, a peptide in both injectable and oral clinical trials is CR845 (Cara Therapeutics, CT, USA), a peripheral kappa agonist targeted at osteoarthritis [79].

Three antimicrobial peptides (AMPs) appear in Table 1 as marketed oral formulations, but these type of agents can be further developed as improved analogues to combat resistance to conventional antibiotics. AMPs typically comprise 15-50 amino acids and contain cationic residues, which allow them to bind to the negatively charged bacterial membrane to initiate death [80]. Compared to regular antibiotics, some AMPs present lower inhibitory concentrations, have broad spectra of activity, and are effective against multi-drug resistant *Staph. Aureus* (MRSA) bacteria [81]. Despite the fact that there are hundreds of AMPs available [82], recent translation of the clinical trials into commercial products has not occurred [80]. AMPs are generally poor candidates due to their low oral bioavailability, however surotomycin (Cubist Pharmaceuticals, Lexington, MA, USA) is an example of a cyclic lipopeptide which is minimally absorbed and is in Phase III clinical trials for oral treatment of *Clostridium difficile* (NCT 01598311, NCT 01597505) [83].

Peptides that are already marketed as injections understandably dominate the field of candidates for potential oral formulation for systemic delivery. From a regulatory perspective, there is the advantage that safety studies will have been completed for the

molecule, at least by the injectable route. On the other hand, molecules developed for injection have been designed with physicochemical features usually unsuited for oral delivery and therefore represent a sub-optimal starting material for oral formulation. Furthermore, peptide doses in oral dosage forms need to be at much higher levels than in injectable counterparts, emphasizing the importance of high potency and a wide therapeutic index to compensate for low oral bioavailability and high intra- and inter-subject variation. This can increase the final product cost, although that can partly be offset by the lack of requirement for sterile products and trained personnel and the lower production costs of peptides in the past 10 years [4].

4. Brief assessment of selected oral peptide strategies in preclinical stages

Strategies to enhance oral delivery of peptides typically include use of permeation enhancers (PEs), enzyme inhibitors, multi-particulate systems, nanotechnology, targeted particulates, colonic delivery approaches, as well as peptide modification [3,16,84]. The most promising strategies applied to oral delivery of peptides by companies at different clinical stages are discussed here. A range of agents including surfactants, bile salts, chelating agents, fatty acids and chitosan have been reported as effective intestinal PEs [85]. Lower bioavailability values are commonly observed after administration by oral gavage of mixtures of drug with PEs in solution compared to results obtained after administration directly into intestinal regions in animal models. This is likely due to dilution and intestinal motility factors, which will impact presentation at the epithelium [86]. Some authors suggest a close correlation between permeation enhancement induced by PEs and intestinal mucosal damage [87]. It is well known that PEs can induce a high level of cytotoxicity in intestinal epithelial cell monolayer studies, but tend to cause less damage to isolated intestinal tissue mucosae at the concentrations required for permeation enhancement [88]. Intact intestinal membranes *in vivo* in

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gavage, perfusion or instillation studies however, are far more resistant to membrane perturbation effects of PEs than in cell culture models or isolated tissue mucosae, likely due to rapid epithelial restitution and recovery and the protective mucus layer present *in vivo* [87,89].

Peptidase inhibition

It is estimated that 40 different peptidases among endo- or exopeptidases are encountered in the GI tract of humans [90]. Cyclic peptides present more resistance against enzymatic cleavage of susceptible peptide bonds due to low structure flexibility, whereas larger linear peptides are more vulnerable to enzymatic cleavage due to the higher number of enzyme susceptible peptide bonds and high structural flexibility [91]. Enzyme inhibitors protect peptides from luminal degradation mainly by two mechanisms: enzyme inhibitors (eg: aprotinin, soybean trypsin inhibitor, FK448, chicken ovomucoid) can bind to the target enzyme and reduce its activity [3], or can locally modulate the pH away from the optimum value for peptidases. Peptidase activity is especially high in the duodenum and upper jejunum, and activities can be minimized in low pH environments [90]. Taking advantage of this, the excipient and pH modifier, citric acid (CA), inhibits intestinal serine proteases and is a useful agent in some oral peptide dosage forms [92].

PEGylation strategies

Combining peptides with polyethylene glycol (PEG) is the most common chemical modification to improve peptide PK due to PEG's biocompatibility and ease of cross-linking to peptides. Site-specific PEGylation of native and recombinant proteins preserves bioactivity and improves efficacy following injections [93]. In addition, it

increases molecular weight, shields proteolytic sites and prolong half-life *in vivo*, which results in increased stability and less frequent administration requirements [94]. Injected PEGylated proteins on the market include growth hormone antagonist (Somavert®, Pfizer, USA), erythropoietin (Mircera®, Roche, Switzerland) and anti-TNF-α Fab (Cimzia®, UCB, Belgium) [42]. For potential oral delivery, peptides conjugated to branched chain PEGs display increased pH and thermal stability and higher resistance to intestinal proteolytic digestion compared to linear PEGs [94]. Chemical modification of the amino acid peptide sequence can also enhance molecule's stability and oral bioavailability. This strategy can insert more hydrophobic amino acids within the peptide backbone [95]. Additionally, improved protection against liver enzyme degradation was demonstrated for a lipidated peptide without loss in biological activity [96] or by reversible/non-reversible conjugation with lipophilic structures that can enhance the transcellular uptake [97,98].

Colonic targeting

There has been increasing interest in targeting peptide and protein drugs to the colon based on evidence for relatively low proteolysis activity in this segment compared to the small intestine [99,100]. Some studies suggest that colonic proteolysis is 20-60 times lower than the proteolysis in the ileum [99]. There is, however, a bacterial concentration of approximately 10^{11} colony forming units/ml colonic lumen of humans with individual variations and some bacteria are capable of producing peptidases [90,101]. In contrast, reduced enzymatic barrier to peptides, mainly degradation proteases, are present in colonic enterocyte membranes compared to those of the small intestine [102]. Thus, peptides that are susceptible to proteolytic degradation in the small intestine may potentially be delivered for either local administration or for systemic absorption from

the colon. On the other hand, the residence time in the colon is approximately 10 times higher than the small intestine, so even though proteolytic activity is lower in the colon *per se*, exposure times of peptides to those enzymes present can potentially be higher. Factors including other regional differences in the thickness of mucus, pH, surface area, and dissolution capacity may all impact on peptide absorption from the colon [103]. There is also evidence that the colonic apical membrane is more sensitive to PEs than the small intestine [10,88]; perhaps this is due to altered plasma membrane composition between colon and the small intestine, since the duodenum has to cope with regular exposure to high mM concentrations of bile salts [104]. A further caveat is that promoting peptide uptake from the bacteria-rich colon on a repeated basis using enhancers and emulsions raises issues of enabling inadvertent pathogen absorption [102]. The following examples highlight technologies in preclinical research that encompass one or more of the above approaches.

Nanoparticle approaches: Polymeric nanoparticles vehicles for several marketed parenteral drugs; these particles typically have a size on the order of 100-200 nm [105,106]. Nanoparticles have some formulation advantages for biotech drugs such as improvement of the oral bioavailability by protecting the payload as well as controlled release to a particular GI region [107,108]. The nanoparticle system of Nanomega (CA, USA) is composed of chitosan (CS) and gamma poly(glutamic acid) (γPGA); one of its potential applications is for oral insulin therapy. Particles were prepared by ionic gelation by mixing CS and γPGA polymeric solutions in presence of a cross-linker, tripolyphosphate, with $MgSO_4$. These nanoparticles appear to be compact, stable over broad pH ranges, and capable of transporting insulin across the small intestinal epithelium [109]. There was a sustained decrease the blood glucose over eight hour in

diabetic rats, with a relative bioavailability of 15% when administered as an oral suspension, and 20% when presented as freeze-dried nanoparticles in enteric-coated capsules [110–113]. The suggested mechanism consists of nanoparticle adherence and infiltration into the jejunal mucus layer whereby they disintegrate due to pH- sensitivity. The hypothesis is that the particles induce transient opening of tight junctions through which insulin permeates [110,114]. A further modification of CS/γPGA nanoparticles consisted of a covalent conjugation of diethylene triamine pentaacetic acid (DTPA) to γPGA. DTPA acts by chelation of metal cations, including calcium and zinc ions, thus causing the disruption of tight junctions, enhancing paracellular permeability and inhibiting proteolytic activity of metallo-peptidases in the intestinal lumen [115,116]. A further advance was the synthesis of CS/γPGA nanoparticles, containing either exendin-4 or insulin, as a combination therapy to control glucose levels in rats with type 2 diabetes. When orally-administered, the combination therapy was more effective than the corresponding monotherapy in achieving optimal glycemic control [117,118]. It remains to be seen if this technology can reach clinical trials.

Targeted nanoparticles: Nanoparticles conjugated with surface ligands can be associated with cell receptors, such as that of vitamin B12, in order to facilitate intestinal absorption, however targeted particle designs are difficult to scale up and translate [105]. Importantly, the control of physico-chemical properties of the nanoparticles have great influence on the amount of particles that can permeate mucous and reach the epithelium (see Lakkireddy *et al* in this Issue). TrabiOral™ (Transgene Biotek Ltd, India): is a platform technology for oral delivery of proteins and peptides using solid lipid nanoparticles. It comprises encapsulation and conjugation with biologically-active ligands to amplify uptake. One of these active ligands is claimed to

be a previously undescribed intestinal transporter in the intestine, and it has been suggested that targeting it may have advantages over targeting vitamin B12 and transferrin receptor, the main ones being the relatively high uptake capacity, low cost and versatility for ligand conjugation. Patent WO2007113665 claims a polymerized lipid nanoparticle system prepared from stearic/palmitic acid, lecithin, poly vinyl alcohol and with wheat germ agglutinin on the surface as targeting ligand [119]. Transgene´s lead project (TBL-1002OI) is an oral insulin formulations and the company has reported sustained hypoglycemia in rats for ~ 10 h following oral dosing. *In vivo* single-blinded efficacy studies were performed in diabetic rats [120,121], but these data have not been subject to peer-review. Recently, Pridgen et al [122] described a poly(lactic acid)–*b*-poly(ethylene glycol) (PLA-PEG) block copolymer-based nanoparticle containing insulin and with Fc-thiol surface ligand groups to target the neonatal FcRn receptor in murine intestine. They achieved hypoglycaemia in wild type mice which was superior to untargeted, but was absent in FcRn knock-out mice.

Among the challenges for oral peptides in nanoparticles, targeted or untargeted, is to produce them in industrial scale, to achieve high peptide loading and to retain stability in intestinal fluids [16]. There is no consensus on whether the optimal peptide-in-nanoparticle design is to promote particle uptake or to trigger release of peptide close to the GI wall (See Malhaire *et al*, this Issue). If it is the former, then at least the rationale of "nano" becomes clearer since particle size, composition, charge and receptor-targeting are important considerations in promoting small intestinal epithelial uptake [123], but in this case, peptide release should not occur in the GI lumen. If it is the latter, then it is hard to argue that "nano" has any inherent advantage over microparticles or other encapsulation approaches and, in this scenario, one can envisage

a key role for PEs where the payload should be entirely released in the small intestinal lumen over a short period and will require assistance to permeate the epithelium. It is clear that nanotechnology is at a very early stage for oral peptides and that only a few prototypes are in clinical assessment in contrast to conventional formulations.

Preclinical PEs: Intravail® is the technology of Aegis Therapeutics (CA, USA). These PEs are a group of alkylsaccharides composed of disaccharides and alkyl chain substituents with lengths between 10 and 16 carbons. Two classes of alkylsaccharides, namely, alkylglycosides (e.g. tetradecyl and dodecyl maltoside), and alkyl esters (e.g. sucrose monododecanoate) are especially interesting, combining absorption enhancement (by allowing controlled transient mucosal permeation by both paracellular and transcellular routes) with a lack of toxicity (GRAS substances) and an effect on preventing peptide and protein aggregation denaturation. For prevention of aggregation, they function like other surfactants in covering exposed hydrophobic sites prone to aggregation with a hydrophilic face [85,124,125]. Although most data concerns their use in nasal peptide absorption enhancement, recent rodent studies have shown that these alkylsaccharide excipients increase oral bioavailability of a number of peptides. Thus, oral delivery of octreotide and the novel anti-obesity/ anti-diabetic leptin-like peptide, D-Leu-OB3, in the presence of Intravail® revealed high systemic bioavailability values in rodents compared to s.c. injection [126,127]. Additionally, efficacy of exenatide or pramlintide formulated with dodecyl maltoside has also been reported in insulin-resistant male C57BLK/6-m db/db mice following oral gavage in the presence of D-Leu-OB3 [128]. Regarding safety, Intravail® excipients metabolize rapidly to the corresponding free sugars and fatty acids or corresponding long-chain fatty alcohols upon administration [124].

Microneedle approaches: The concept of Rani Therapeutics (CA, USA) is a capsule (also referred to as "robotic pill") that consists of two chemical compartments filled with CA and sodium bicarbonate, respectively. As the capsule travels down the GI tract, it remains intact until the pH increases to 6.5-7.0, where the barriers between the two substances erode and mix, creating a chemical reaction that pushes drug coated sugar-based micro-needles through the outer layer of the capsule to penetrate the epithelium. The technology is in preclinical studies, claiming more than 50% bioavailability for insulin and adalimumab in Press Releases [129–131]. Massachusetts Institute of Technology (MIT) researchers reported on a similar device, also capable of potentially delivering peptides including insulin and tested in pigs. Instead of sugar, their "needle pill" used stainless steel that was gradually exposed as pH levels in the digestive system wore off the capsule's outer layer [132]. It will be interesting to see if such designs can be scaled for man and more importantly, if they can negotiate the extensive toxicology questions raised by such a direct interaction with the intestinal wall.

5. Assessment of oral peptide technologies at clinical stages for systemic delivery

5.1 Phase I

Shanghai Biolaxy (China) has an oral peptide/protein delivery technology sourced from NOD Pharmaceuticals Inc. (China). This capsule technology consists of bioadhesive calcium phosphate nanoparticles, which are enteric coated with cellulose acetate phthalate. The peptide is mixed with calcium phosphate in the presence of PEG salts of fatty acids or bile salts as precipitating agents [4,133]. Nodlin™ basal insulin is the lead candidate and its feasibility in human subjects has been tested in Phase I studies (ChiCTR-TRC-12001872) [134]. PK and PD profiles of Nodlin™ were evaluated in 12 healthy volunteers receiving one of three oral doses (50, 100, or 200 U) or a neutral protamine- Hagedorn (NPH) insulin administered by the s.c. route (6 U) on different

dosing days. Enteric-coated insulin capsules were well-tolerated and induced a uniform metabolic effect that lasted for at least 6 hours, Both the duration of action and T_{max} suggested that the plasma glucose reduction induced by the oral formulation was similar to that obtained for s.c. However, issues were the high variability in AUC, inability to detect minimal insulin concentration changes, and the fact that administration was restricted to fasting subjects [134]. Shanghai Biolaxy are also researching oral exenatide with the NOD technology in preclinical studies [135].

Oshadi (Israel) has developed an oral carrier (Oshadi Icp) that is claimed to enable absorption of peptides from the gastrointestinal tract (Fig. 4). Patent US8936786 B2 states that the vehicle is a particulate mixture of pharmacologically inert silica nanoparticles (~100 nm) with an adsorbed polysaccharide (either branched or unbranched), and a biologically active protein or peptide, which are then suspended in an oil (natural- e.g. sesame/olive or synthetic – silicone) [16] and incorporated to enteric capsules. The safety and preliminary efficacy for oral insulin was evaluated in T1D subjects in a Phase Ib trial in 2013 (NCT01772251), although the data has not been disclosed. Currently, Phase II studies are being conducted (NCT01973920) in order to evaluate the safety and feasibility of multiple oral administrations in patients. The study will include four weeks of multiple-dose administration of Oshadi's oral insulin to determine efficacy, safety and PD effects; completion is expected in 2016. The rationale behind the technology seems to be primarily one of insulin protection, however there is no data in the public domain as to whether the particles are taken up by the epithelium, or if silica plays a specific enabling role.

5.2 Phase II

Merrion Pharmaceuticals Ltd. (Dublin, Ireland) has developed a gastrointestinal permeation enhancement technology (GIPET®), which is an oral solid dose technology platform based on medium chain fatty acids (MCFAs) salts, and their derivatives in order to enhance the drug absorption from the small intestine (Fig. 5) [3]. Originally licensed from Elan Drug Technologies (Dublin, Ireland), the technology is primarily for low permeability molecules such as peptides, but can also improve oral bioavailability of some poorly permeable small molecules including acyline and bisphosphonates. It is thought to result in lower intra- and inter-subject variation and improved PK profiles. Although collectively referred to as GIPET®, Merrion's proprietary formulations consists of three different enteric coated formats, all of them tested in humans: GIPET® I is an enteric coated tablet consisting of a medium chain fatty acid (MCFA) in powder form combined with the drug in selected ratios by weight. GIPET® II is a microemulsion pre-concentrate of oil and surfactant with the active in an enteric coated gel hard or soft capsule, while GIPET® III is a mixture of fatty acid derivatives in an enteric coated gel capsule [137]. To our knowledge, Merrion's published preclinical and clinical data refers solely to GIPET® I tablets. The prototype MCFA is sodium caprate (C_{10}), which has had extensive prior use as a food additive [85], but it acts as a mild surfactant at the high 150 mM doses used in GIPET® I *in vivo*, increasing apical membrane fluidity to non-specifically allow both transcellular and paracellular transport of the active. *In vitro*, lower mM concentrations of C_{10} primarily increase paracellular transport by contracting cytoskeletal actin filaments leading to the opening of bicellular tight junctions by reducing claudin 5 expression and of tricellular ones by reducing that of tricellulin [138]. GIPET® materials form mixed micelles above their critical micellar concentration (CMC) and multi-lamellar vesicles at still higher concentrations, with a

likely interaction with bile salts in the upper jejunum. The hypothesis is that maintaining concentrations of C_{10} at the epithelium above its CMC causes an increase in permeation, as long as the payload is also co-released in high concentrations in order to maintain a gradient. Though the micelle/vesicle structures may not penetrate biological membranes *per se*, they may permit surface attachment of peptides leading to access to endocytotic pathways through the plasma membrane. For further discussion of the mechanisms of action of C_{10} and other PEs, see Maher *et al.* in this Issue.

Merrion has partnered with Novo Nordisk A/S (Denmark) to develop selected insulin and GLP-1 agonists using GIPET®. Five Phase I clinical trials (NCT02470039, NCT02304627, NCT01931137, NCT01796366, and NCT01334034) were conducted with GIPET® oral insulins (NN1953, NN1954, and NN1956) to treat T1D and T2D; three Phase I studies (NCT02094521, NCT01967589, and NCT01405261) were carried out with a GLP-1 analogue (NN9928) to treat T2D In 2015, Novo initiated the first Phase IIa proof-of-principle clinical trial with GIPET® and their long-acting insulin analogue (NN1953), as well as a Phase I trial with a new oral insulin analogue (NN1957). Regarding Novo's GLP-1 analogue oral formulation, a Phase I trial aimed at investigating the safety, tolerance and PK was completed in 2014 [139]. Concerning overall GIPET® safety, the short term trials to date indicate that GIPET-based products were administered safely to some human subjects on a repeated basis despite mild-to-moderate GI side-effects.

Oramed Pharmaceuticals (Jerusalem, Israel) has developed its proprietary Protein Oral Delivery (POD™) technology. It is composed of an enteric-coated capsule containing an oily suspension of the peptide or protein, an enzyme inhibitor (e.g. soya bean trypsin inhibitor and/or aprotinin) and PEs such as EDTA or bile salts (both possibly doubling up as protease inhibitors), suspended in omega-3 fatty acids [4,140]. The precise

compositions used in any particular clinical trial are not in the public domain. Oramed´s lead candidate is an oral pre-prandial rapid-acting recombinant human oral insulin (ORMD-0801). Optimal ratios of adjuvants to insulin in initial safety profiling were based on results from a small Phase I study performed with five different formulations in a total of eight patients in 2010 [141]. In a succeeding study, the pre-prandial oral insulin alongside patient's daily s.c. insulin regime was adjudged safe and well tolerated and yielded a 17% reduction in glycemia in a small cohort of uncontrolled T1D patients (NCT00867594) [142]. ORMD-0801 has recently completed two Phase IIa clinical trials in T1D and T2D patients, respectively (NCT01889667, NCT02094534). In the T1D Phase IIa study designed to examine exogenous insulin requirements, ORMD-0801 capsules given before meals appeared to be safe and well-tolerated (according to the Company), with trends observed for a decrease in rapid acting insulin, a decrease in post-prandial glucose, a decrease in daytime glucose (continual monitoring) and an increase in post-prandial hypoglycemia [143]. In the Phase IIa trial investigating ORMD-0801 in thirty T2D patients, a Press Release claimed that it was also well-tolerated and that there were trends for decreases in blood glucose and well-defined short-term increases in plasma insulin [144]. ORMD-0801 is currently undergoing a double-blind, randomized Phase IIb study for T2D in 180 patients, designed to generate sufficient data for efficacy and safety endpoints (NCT02496000) [145]. Oramed is also developing an oral GLP-1 analog capsule (ORMD-0901), which is under preclinical investigation [146]. Although Oramed´s POD™ technology approach is aggressively moving through clinical phases, as with all technologies using PEs, there are unknown safety effects of chronically increasing intestinal permeability, as well as additional safety considerations associated with high doses of oral EDTA and peptidase inhibitors. It is unclear how this technology

fundamentally differs from others that also incorporate protease inhibitors and PEs in an enteric-coated capsule. Any claim for differentiation over competing technologies must therefore be on the basis of optimization and scale up of the formulation using defined ratios of the key constituents.

Proxima Concepts (UK) has developed a proprietary oral peptide delivery technology, Axcess™. The system is based on an oral capsule containing the protein/peptide, as well as stabilizers, GRAS-listed aromatic alcohols as PEs (at least 25% by weight), and solubilizers to improve transcellular absorption [3,147]. Capsulin™ is the oral insulin candidate developed by the subsidiary, Diabetology, Ltd., and consists of a standard enteric-coated capsule that dissolves rapidly in the small intestine bringing insulin and excipient components into contemporaneous contact with the intestinal cell wall. Safety and efficacy of Capsulin™ was investigated in Phase II clinical trials (EudraCT number: 2005-004753-95), reporting positive results and a good safety and tolerability profile. Administration of Capsulin™ oral 150 and 300 U insulin doses to 16 T2D patients demonstrated a hypoglycaemic action over a period of a 6 h glucose clamp procedure. This was claimed to reflect high target portal vein insulin concentrations, but not those of peripheral plasma insulin, which is regarded as a physiological and safety advantage [148,149]. It is not in the public domain as to whether Capsulin™ is progressing further or is partnered with Pharma companies. Additionally, a second Proxima subsidiary (Bone Medical Ltd) synthesized other peptide formulations: Capsitonin™ (salmon calcitonin) and CaPTHymone™ (PTH), also referred as BN002 and BN003, which completed Phase IIa and Phase I, respectively for the treatment of osteoporosis. It seems however, that neither prototype remains in development [150].

Diasome Pharmaceuticals Inc. (OH, US) has developed a proprietary oral nanoparticle targeting technology, hepatic-directed vesicle-insulin (HDV-I) (Fig. 6). This is an lipid system aimed at reestablishing the normal insulin physiological responses in the liver in T1DM or T2DM patients through hepatocyte-targeting [151]. The HDV-I consists of insulin incorporated into a <150 nm lipid nanoparticle attached to a hepatocyte-targeting ligand (biotin-phosphatidylethanolamine). The targeting ligand facilitates capture of HDV-I by hepatocytes following uptake from the intestine through the hepatic-portal vein after oral administration [152]. High efficacy of peripherally-infused HDV-I compared to regular insulin was previously demonstrated in a hepatic glucose balance study in diabetic dogs. HDV delivered insulin to the liver efficiently, while promoting hepatic glucose uptake with a potency that was 100-fold greater than that of the same dose of injected regular porcine or human recombinant insulin [152]. A number of small scale clinical trials were conducted for oral HDV-1 in low numbers of T1DM and T2DM patients (NCT00521378 and other non-registered trials) [153,154].   These demonstrated significantly improved glycemic control during an oral glucose tolerance test following oral administration of HDV-I in an oral gelatin capsule using a low dose of insulin (5 IU). Treatment of patients with oral HDV-I was safe and well tolerated. In 2009, Diasome started a large Phase II/III 18 week study in the US in 230 T2DM patients (NCT00814294). The primary end-point was to compare reductions in mean glycated hemoglobin levels (HbA1c) between two doses of oral HDV-I (5 U and 15U) and placebo in patients on a background of oral metformin. The recruitment status and results were never updated on the Clinical Trials registry. The development stage therefore remains at Phase II for oral HDV-I [155].

Sigmoid Pharma Ltd (Dublin, Ireland) has developed a single-multiple pill (SmPill®) technology. It attempts to address solubility, permeability, active stability and regional targeting. SmPill® may therefore be suitable for a wide range of drug classes such as soluble and insoluble drug small molecules, poorly permeable molecules and larger molecules including proteins and peptides. The multi-particulate nature of SmPill® allows for the uniform distribution of drug within the GI tract which, coupled with appropriate coating, can release to the optimal site. Seamless minispheres are formed by thermotropic gelation of an oil in water (O/W) emulsion extruded into oil. Targeted drug release to different GI regions can be achieved through application of suitable polymer coatings. The final dosage format comprises SmPill® minispheres (uncoated or coated) filled into hard capsules or sachets [156]. The flexibility of the technology permits the incorporation of PEs, pH modulators, and enzyme inhibitors. Sigmoid's process is mild to peptides, and provides further protection due to the shielding effect of the emulsion format and uncoated beads [157,158]. Preclinical assessment of a SmPill® formulation containing sCT demonstrated the ability of this technology to promote systemic absorption of the peptide by colonic administration in rats [157]. sCT was loaded into SmPill® minispheres combined to a range of PEs, and instilled into rat colon. Hypocalcaemia was achieved, indicating the bioactivity of sCT *in vivo*, and 3-fold enhancement in sCT absolute bioavailability was observed to some formulations over sCT solution instilled into the same segment [157]. In a porcine study, SmPill® minispheres were used to deliver orally cyclosporine A to the colon in order to design a local delivery system for mild-to-moderate ulcerative colitis that would concentrate the peptide in the damaged epithelium rather than to promote systemic absorption, with its attendant nephrotoxic side-effects [158]. This technology has completed a Phase 2 clinical trial by delivering cyclosporine A to the colon for the local treatment of

ulcerative colitis (NCT01033305) according to company information [159], and the results are awaited.

5.3 Phase III

Biocon Ltd (Bangolore, India) is developing IN-105, an orally conjugated insulin originated from the modification of hexyl insulin mono-conjugate 2 (HIM2), acquired by take-over of Nobex (North Carolina, USA) [160]. IN-105 is a modified human insulin in which a single short-chain amphiphilic oligomer is covalently linked by a non-hydrolysable amide bond to the free amino acid group on the Lys-β29 residue of recombinant human insulin. The amphiphilic oligomer is a polyethylene glycol (PEG) derivative, specifically methoxy-triethylene glycol propionyl, which is mainly responsible for the increase in water solubility of the insulin analogue. The alkylated PEG also confers improved stability against enzymatic degradation, probably due to steric hindrance [161,162]. The most advanced formulation for IN-105 was a second-generation tablet, claimed to be simple to manufacture, uses readily-available excipients, and has good stability at ambient conditions [163]. Regarding its mechanism of action, IN-105 has similar insulin receptor binding and metabolic activity to that of human insulin, and is thought to have improved permeability and half-life in the GI tract, and it retains similar pharmacodynamics, safety and clearance as native insulin [162,163]. IN-105 reduced post-prandial glucose excursions by 2 h in a dose-dependent manner and was well tolerated by patients in Phase II trials (CTRI/2009/091/000479) [162]. In Phase III studies conducted in India in T2D patients however, IN-105 did not meet its primary endpoint and failed to lower the level of plasma HbA1C by 0.7% compared to placebo. Secondary endpoints were met in that IN-105 behaved in part like prandial insulin by reducing blood glucose levels during and after meals

(CTRI/2008/091/000276). However, Biocon is now further developing this prototype in Phase II studies partnered with Bristol-Myers Squibb (NJ, USA) [164].

Emisphere Technologies (NJ, USA) has a library of low MW *N*-acylated alpha-amino acids with intestinal PE properties, Eligen®. Sodium N-[8-(2-hydroxybenzoyl) aminocaprylate] (SNAC or salcaprozate sodium) is the lead carrier, having achieved GRAS status [84,165]. The other two lead agents are 8-(N-2-hydroxy-5-chloro-benzoyl)-amino-caprylate (5-CNAC) and monosodium N-(4-chlorosalicyloyl)-4-aminobutyrate (4-CNAB) [84]. The Eligen®-associated poorly permeable actives are not conjugated since the delivery agents interact with them to create a weak non-covalent association. Carriers are blended with the API using standard pharmaceutical processes, which makes manufacturing dosage forms simple, economic and easy to scale. The technology appears suitable for a range of dosage forms: tablets and capsules, as well as solutions and suspensions [165]. Regarding the highly controversial Eligen® mechanism of action, the lipophilic molecule-carrier complex is claimed to enable transcellular absorption neither by regulating tight junctions nor by causing significant perturbation to the intestinal epithelium. The complex is hypothesized to dissociate upon permeation by an un-deciphered mechanism. In the case of peptides, the combination of carrier and peptide forms an insoluble entity at low pH values, thereby reducing the peptide's susceptibility to degradation in the stomach and duodenum. At the higher pH found in the jejunum, the complex dissolves but apparently remains covalently-attached until it crosses the apical membrane [166]. In most examples, absorption of both payload and carrier is rapid with T_{max} typically within an hour of dosing [85,165].

Nordic Biosciences A/S (Denmark) and Novartis A/S (Switzerland) conducted up to three Phase III clinical trials (NCT00525798, NCT00486434, NCT00704847) using an oral tablet of 5-CNAC with sCT. Two of these studies were performed towards evaluating symptom efficacy, safety and tolerability of the formulation in 2000 osteoarthritis patients with moderate to severe knee pain and joint structural damage, a possible new indication for sCT. However, at the 24 month endpoint there was no significant joint-narrowing or pain-relief effects in the two studies. The adverse reactions to the formulation were mainly mild-to-moderate and GI associated: diarrhea, nausea and vomiting; these disappeared spontaneously when treatment was stopped. These events occurred in 40-46% of oral sCT: 5-CNAC-treated subjects versus 26-30% in placebo groups [167]. A Phase III trial was also performed by Novartis and Nordic Biosciences to assess the safety and efficacy of sCT in the treatment of post-menopausal osteoporosis, the clinical indication for nasal and injected sCT. Although published results are not publically available, the company released an interpretation of the data indicating that the study failed to demonstrate a significant treatment effect on the reduction of the occurrence of new vertebral fractures after three years, the primary endpoint. In addition, no significant response was observed on the key secondary endpoints: new non-vertebral fractures or new clinical fractures, although again the oral sCT displayed a positive safety profile [168].

Since the late 1990s, Emisphere made efforts to develop an oral insulin and carried out a an unpublished Phase II study (2006), but development was thereafter suspended [163]. However, Emisphere have been collaborating with Novo Nordisk since 2010 to develop Eligen® in combination with a selection of Novo-Nordisk's GLP-1 receptor agonists and insulins [169]. Novo Nordisk announced in 2015 that it had successfully completed a Phase II trial for an oral formulation of its long-acting GLP-1 analogue, semaglutide

(NN9924; OG217SC), with SNAC. The study was performed in ~600 T2DM patients for 26 weeks, to investigate dose range, escalation, initial efficacy, and safety of once-daily oral semaglutide [170]. Plasma Hb1AC levels were reduced by up to 1.9% and it appeared to have a safe and well tolerated profile in data reported by the company, although GI-related adverse events were reported in some subjects. In different clinical studies these adverse events appeared to be drug dose-dependent and corresponded to those reported for other forms of the active molecule [84,170]. Since Eligen® formulations are rapidly absorbed, metabolized and eliminated and they do not accumulate in the organs and tissues, there appeared to be no major safety issues at those dose levels and dosing regimens in these relatively short term studies. Moreover, in respect of safety, the first Eligen®-based product recently reached the market: an oral vitamin B12 using 100mg SNAC, yielding 5% absolute bioavailability [171]. To put the recent clinical data in context however, since pharmocodynamic equivalency was seen with 1 mg semaglutide by s.c. injection and oral doses of up to 40 mg, this suggests very low single digit relative oral bioavailability. One interpretation is that Eligen® is performing on a par with its previous record, but perhaps the difference now is its combination with newer oral peptide candidates with higher stability and a longer $t_{1/2}$. Novo-Nordisk have initiated an extensive Phase IIIa programme with this technology and are undertaking six safety and efficacy dose-ranging studies (PIONEER) in 2016 [172].

Enteris Biopharma Inc. (NJ, USA) has an oral delivery technology, Peptelligence™, which originated at Unigene Laboratories (NJ, USA). It consists of a Eudragit® L30 D-55-coated tablet designed to dissolve at the duodenal pH after passing intact through the pyloric sphincter. The enteric coat may diminish the effect of food or liquid on peptide

absorption [84]. It has a sub-coat tablet layer, which both protects the core from moisture and the enteric coating from acid excipients in the core. The peptide is enclosed in the core tablet, in a lyophilized format, compressed together with maltodrexin-coated citric acid (CA) granules. The coating of the acidic granules protects the peptide from degradation under acidic conditions by avoiding direct contact with other tablet components. Secondly, the CA protects the peptide from proteolysis in the lumen after release by temporarily lowering the local pH creating a GI microenvironment [84]. However, a recent study confirmed there is no PE effect of CA since it is a weak calcium chelator at acidic pH values, so this molecule acts primarily as a pH modulator to decrease activity of serine proteases [92]. In keeping with the approach of many of the technologies discussed above, co-entrapped acyl carnitine PEs are thought to be co-released in high concentration with payload close to the jejunal epithelium in order to generate efficacy [173,174]. This technology has been in advanced clinical trials for three different peptides.

Tarsa Therapeutics (PA, USA) was formed to bring Enteris's sCT formulation to market. The formulation achieved safety and efficacy endpoints for a Phase II trial (NCT01292187) in postmenopausal women with low bone mineral density (BMD) and increased fracture risk from osteopenia [11,15], and then for a Phase III trial on oral sCT in postmenopausal osteoporosis (ORACAL; NCT00959764) (Fig. 7). Both trials were carried out with TBRIA™ tablets (previously Ostora™). The oral formulation is composed of a core containing 200 μg (1200 IU) of recombinant sCT combined with 500 mg of CA in a Peptelligence™-based design [15]. Thus, although the use of PE may improve sCT oral bioavailability [157], it seems that no PE is present in TBRIA™, and that enzymatic inhibition caused by co-release of CA and sCT was sufficient to

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achieve PD effects on a par with nasally-delivered sCT, and this would be consistent with an estimate of 1% oral bioavailability for this potent peptide in this formulation [175]. A new drug application (NDA) for TBRIA™ tablets was recently submitted to the FDA to treat osteoporosis in women >5 years post-menopause as a second-line treatment, signaling the possibility of the first oral sCT tablet to reach the market despite the negative publicity surrounding putative cancer risks from chronic sCT in such patients, despite lack of causal evidence to date [14,176]. The fact that TBRIA™ does not contain PEs may alleviate safety concerns of the regulators over chronic administration of PEs in this first submission for Peptelligence™.

An oral recombinant human PTH fragment [rhPTH(1–31)NH_2] is another peptide orally formulated by Peptelligence™. rhPTH(1–31)NH_2 is a linear amino acid sequence [177], however its larger MW compared to sCT may further limit its permeation and therefore it requires PEs in addition to enzymatic degradation prevention. Thus, this oral formulation was prepared by incorporation of rhPTH(1–31)NH_2 in a tablet by direct compression of the dry blended peptide (5 mg), coated CA granules (pH modulator) and lauroyl or palmitoyl carnitine [178]. A Phase II trial originally sponsored by Unigene (NCT01321723) included 97 women in a postmenopausal period and diagnosed with osteoporosis. Significant increases in lumbar spine bone mineral density was observed for oral rhPTH(1–31)NH_2 administered once daily after 6 months compared to baseline values and PK and safety endpoints were also achieved [178,179]. A curiosity is that this peptide is not the same as the approved injected rhPTH (1-33) (teriparatide) and would be classified as an NCE.

Cara Therapeutics (CT, USA) is also working with Peptelligence™ to formulate a peripherally-selective kappa opioid receptor agonist targeted at peripheral pain-sensing

nerves, CR845 [79]. Because of its D-stereochemistry, this peptide is resistant to metabolism by intestinal proteases [180]. The oral formulation was administered to 50 healthy volunteers in a Phase I trial and oral bioavailability was claimed to be 16% [174]. No information regarding specific formulation components were disclosed, but the formulation was considered safe and well tolerated [174]. Recently, a Phase II trial (NCT02524197) was publicized by Cara, aiming primarily to assess the safety and tolerability of orally-administered CR845 in patients with osteoarthritis of the hip or knee. Secondary endpoints include PK profiling and efficacy from tablets administered twice daily over two weeks at 0.25, 0.5, 1 and 5 mg dose levels of CR845.

Chiasma Ltd (Israel) is the developer of Transient Permeability Enhancer (TPE®), which the MCFA, sodium caprylate and the peptide are combined as part of an aqueous phase, and then mixed with oil-based surfactants to form an oily suspension. The combined components appear to act in part by opening the tight junctions to temporarily increase small intestinal permeability of peptides [181]. Octreotide is a cyclic hydrophilic and potent octapeptide (MW ~1.0 kDa), analogue of human somatostatin [182]. It is poorly permeable, but has reasonable stability against peptidases [183]. Therefore, commercially available octreotide products include a s.c. daily injection and a 1-month sustained-release octreotide depot for i.m. injection with a large gauge needle [182]. It has orphan drug designation for treatment in acromegaly patients, which stimulated commercial interest in creating an oral version [184]. The oral formulation (Octeolin™) consists of a hydrophilic fraction (octreotide acetate, polyvinyl pyrrolidone (structural element), sodium caprylate (PE) and water (solvent)) that after dissolution is further lyophilized and suspended in a lipophilic medium (the non-ionic surfactant, Polysorbate® 80, as well as glyceryl monocaprylate and glyceryl tricaprylate) [181]. The

oily suspension is transferred to hard gelatin capsules that are subsequently enteric-coated with a 20% aqueous suspension of Acryl- EZE® [181] (Fig. 8). Four crossover open-label clinical trials carried in 75 healthy volunteers, revealed a dose-dependent PK profile for Octreolin®, as well as a significant PD effect measured as reduced growth hormone secretion compared to baseline [182]. Plasma concentrations of Octreolin® loaded with 20 mg of octreotide were comparable to 0.1 mg s.c. octreotide, indicating a relative oral bioavailability of 0.5% [182]. In a pivotal Phase III trial (NCT01412424), there was evidence that this oral bioavailability was still enough to reduce plasma hormone biomarkers as an alternative to monthly i.m. injection [185]. The trial comprised a lead-in phase of 2-5 months for dose escalation in 155 patients (20, 40, 60 or 80 mg Octreolin®) in order to control insulin growth factor (IGF)-1 and growth hormone levels and acromegaly symptoms, followed by a fixed dose 6 month period for initial responders. There were positive endocrine effects for 65% of the participants in the first phase and for 62% across the fixed dose period over a total of 13 months, accompanied by adequate safety and tolerability [185]. Chiasma filed an NDA for Octreolin® (now Mycapssa™) in 2015 for the maintenance therapy of adult patients with acromegaly [186].

Synergy Pharmaceuticals Inc. (NY, USA) created two peptide analogues from naturally occurring human uroguanylin, which plays a key role in regulating normal GI fluid secretion by activation of guanylate cyclase C receptors in a pH-dependent fashion to induce salt and water secretion to normalize bowel movements in order to treat irritable bowel syndrome of the constipated sub-type [187,188]. Plecanatide is a hydrophilic 16 amino acid peptide (~1,6 kDa) cyclic, with two disulfide bonds, presenting a structure virtually identical to that of uroguanylin except for the replacement of the penultimate

aspartate on N-terminus with a glutamate amino acid [189]. Dolcanatide, presents a glutamic acid instead of a an aspartic acid at position 3 on N-terminus, and d-stereoisomers of aspargine and leucine at positions 1 and 16 respectively to enhance stability [189]. Both peptides are part of library of guanylate cyclase-C ("GCC") agonist peptides, and are under different clinical phases [187]. Oral formulation of tablets containing a range of concentration of the therapeutic peptides are based on direct compression of APIs and inactive ingredients into enteric-coated tablets [187]. Plecanatide is in advanced stage clinical trials for chronic idiopathic constipation (CIC) and irritable bowel syndrome with constipation (IBS-C). Two Phase III clinical trials (NCT02122471, NCT01982240) assessed efficacy and safety of two different plecanatide treatment dose level (3.0 mg and 6.0 mg), taken as a tablet once-a-day in patients with CIC over 12 weeks. The number of patients durable overall responders were significant higher than placebo [190]. A Phase III open-label extension study of plecanatide for CIC (NCT01919697) is ongoing to evaluate safety and tolerability over 52 weeks, final data collection is expected to December of 2015. Plecanatide for IBS-C is at Phase III trials (NCT02387359, NCT02493452) evaluating plecanatide oral tablets with 3.0 and 6.0 mg doses, following positive results obtained in a Phase IIa (NCT01722318) in terms of increase complete spontaneous bowel movement frequency and significantly reduced abdominal pain [191]. The company claims that dolcanatide has successfully completed a phase II (NCT01983306) study in patients with opioid-induced constipation (OIC), however the data was not published yet [192]. Dolcanatide is also under investigation for treatment of ulcerative colitis (UC) [193]. Uroguanylin analogues require local regional delivery and access to apical receptors on the brush border membrane, so this is not the same challenge as for systemic delivery. Fig. 9 summarizes current clinical progress of oral peptides to date over all stages. In making

assessments of the clinical data, we note that many of the companies claiming clinical progress have not actually published the data in peer-reviewed journals or have even have updated the Clinical Trials.gov website, so accurate interpretation is difficult; this problem was also remarked upon in a recent oral insulin review [194]. Finally, we note that a similar but complementary review [195] has just been published following our submission; its focus was primarily on oral macromolecule formulation compositions, but with a greater emphasis formulations designed for localization in intestinal regions and less on the clinical aspects.

6. Conclusions

A range of biologically-active injectable peptides have been produced during the last three decades indicating that peptide-based medicines are useful treatments for many intractable diseases. Clinical studies suggest that peptides are potent, specific and safe and they comprise an increasing proportion of molecules in clinical trials. Modern methods of peptide preparation by recombinant or synthetic routes allow production on a larger scale and at more reasonable cost than 10 years ago. In order to reduce regulatory risk from working with NCEs, most Pharma company efforts to produce oral delivery formulations are restricted to peptides that are typically on the market delivered by parenteral routes. Translation of approved injectable peptides into oral formats lowers risk of working with an agent that is not a new chemical entity, although safety still has to be proven for the new administration route. The downside is that injectable peptides are designed by medicinal chemists for those routes and they therefore represent an unpromising starting point for oral formulation. Furthermore, companies prefer to include excipients in oral formulations that are already on the market as food or pharmaceutical ingredients, or excipients with GRAS status, but these may not be the most optimal PEs or peptidase inhibitors. Therefore, both strategies understandably lead

to a scenario in which advanced oral peptide clinical trials use neither novel peptides nor novel excipients. On the other hand, the most advanced technologies are generally simple to produce and to scale up and examples are included in a summary of the all the approaches (Fig. 10). Among the technologies in most advanced clinical phases, common formulation strategies are enteric coating, use of PEs alone or combined with pH modulation and peptidase inhibition in emulsions-in-capsules or as solid-dose matrix tablets. The two currently most advanced oral formulations submitted as NDAs in 2015 offer only 0.5 % oral bioavailability for octreotide (Mycappsa™) [182], and a pharmacodynamic equivalence for oral sCT (TBRIA™) with a marketed nasal sCT [11], which is estimated to have a bioavailability of < 1% by that route [196] (note that no PK has been published on the clinical studies of TBRIA™). If both are eventually approved, these should be regarded as niche products since those peptides are exceptionally potent. It remains to be seen if this number can be increased by current and future delivery technologies to levels approaching the 5% that could be more acceptable for other peptides including insulin and GLP-1 analogues. Finally, with exciting new cyclic stable peptides emerging with MW of <1000 Da, perhaps there will be more suitable peptide payloads for oral formulation scientists to work with, and not just "hand-me-down" parenteral peptides?

Figure legends

Fig. 1. The physical barrier of the intestinal epithelium to passage of peptides from the gut lumen to the basal lamina propria. Paracellular spaces are sealed by tight junctions on the plasma membrane. Molecules may cross the cell membrane barrier by different mechanisms: (A) active transport, (B) transcellular diffusion, (C) paracellular diffusion. Peptides of low MW can traverse via tight junctions, but di- and tri-peptides can also be translocated using the hPEPT1 carrier on the apical membrane (adapted with permission from [16]).

Fig. 2. PK profiles for Neoral® and Sandimmune® in children after liver transplantation (mean dose: 19.6 mg/kg/ day, n = 8). Adapted with permission from [26].

Fig. 3. Four possible methods for peptide macrocyclization. Adapted with permission from [70].

Fig. 4. Schematic of Oshadi's peptide nanocarrier. Adapted with permission from [136].

Fig. 5. Cartoon of GIPET® proposed mechanism of action. Adapted with permission from [137].

Fig. 6. Oral HDV-I schematic. Adapted with permission from [151].

Fig. 7. Change in lumbar spine bone mineral density in postmenopausal women with low bone mass and over 54 weeks of a Phase III trial (NCT00959764). Recombinant sCT (rsCT) TBRIA^{TM} tablets or placebo tablets were used. The 1.14 % difference between groups was significant (p=0.027). Adapted with permission from [15].

Fig. 8. Chiasma's oral octreotide formulation. Adapted with permission from [181].

Fig. 9. Oral peptide technologies at different stages of development.* Local intestinal delivery only.

Fig. 10. Schematic of the main peptides under development for oral delivery, along with strategies applied to promote absorption and matched to stage of development.

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Table 1. Commercially available oral peptides

Company	Product(s)	Peptide	Indication	Strategy
Novartis AG (Switzerland)	Neoral®/ Sandimmune®	Cyclosporine	Immunosuppression	SNEDDS, systemic delivery
Ferring Pharmaceuticals (Switzerland)/ Generic (e.g. Actavis Labs FL Inc., NJ, USA)	DDAVP® Tablets DDAVP® Melt Minrin®	Desmopressin acetate hydrate	Central *Diabetes Insipidus*, Primary Nocturnal Enuresis	Chemical modification, systemic delivery
Mitsubishi Tanabe Pharma Corporation (Japan)	Ceredist® Ceredist OD®	Taltirelin hydrate	Spinocerebellar degeneration	Chemical modification to avoid enzymatic hydrolysis, systemic delivery
Theranaturals Inc. (ID, USA)	Reduced L-Glutathione	Glutathione	AIDS-related cachexia/cystic fibrosis	none
Acatavis, Inc. (NJ, USA) /Ironwood Pharma, Inc. (MA, USA)	Linzess® (USA) Constella® (Europe)	Linaclotide	Irritable bowel syndrome, Chronic idiopathic constipation	Acts locally on luminal surface of intestinal epithelium
ANI Pharmaceuticals, Inc. (MN, USA)	Vancocin®	Vancomycin HCl	Infection	Acts locally by inhibition of cell-wall biosynthesis.
Biocon Ltd. (India)	Koolistin®	Colistin sulfate	Infection	Acts locally to SOD and SDD
Several	Several brands (lozenges)	Tyrothricin	Pharyngitis	Acts locally on the throat

Table 2. Examples of oral peptides technologies in preclinical studies

Company	Technology/Product	Peptide(s)	Strategy to promote absorption
NanoMega Medical Corp. (CA, USA)	Nanomega's nanoparticulate system	Insulin Exendin-4	Nanoparticles
Transgene Biotek Ltd. **(India)**	TrabiOral^{TM}/ TBL-1002OI	Insulin	Solid lipid nanoparticles, targeted
Aegis Therapeutics, LLC. (CA, USA)	Intravail$^{®}$	Octreotide/ D-Leu-OB-3 (Leptin)/ PTH/ GLP-1/ AFpep	PE: alkylsaccharides
Rani Therapeutics, LLC. (CA, USA) **Novartis AG (Switzerland)**	Robotic pill	Insulin	Micro-needles/ Local pH modulator: citric acid

Table 3. Oral peptide formulations in current or completed Phase I (*) or II (**) trials

Company	Technology/Product	Peptide	Strategy to promote absorption
NOD Pharmaceuticals, Inc. (China)	NOD/ Nodlin™	Insulin*	Bio-adhesive nanoparticles
Oshadi Drug Administration Ltd. (Israel)	Oshadi Icp	Insulin*	Silica-based nanoparticles
Merrion Pharmaceuticals Ltd. (Ireland) with Novo Nordisk A/S (Denmark)	GIPET®/ NN1957/ OI338GT (NN 1953) OG987GT (NN9926)/	Insulin** Insulin** GLP-1 analog *	PE: sodium caprate
Oramed Pharmaceuticals, Inc. (Israel)	POD™/ ORMD 0801	Insulin**	PEs: EDTA, bile salts Enzyme inhibitors: soy bean trypsin inhibitor, aprotinin
Proxima Concepts Ltd (UK)/ Bone Medical Ltd (Australia)/ Diabetology (UK)	Axess™ / Capsulin™/ Capsitonin™ (BN002)/ CaPTHymone™ (BN003)	Insulin** sCT** PTH*	PE: aromatic alcohols
Enteris Biopharma, Inc. (NJ, USA)/ with Cara Therapeutics (CT, USA)	Peptelligence™	rhPTH (1-31) NH$_2$**/ Cara's CR845*	PE. Acyl carnitine/ pH modulator, CA/ Peptide with D-stereochemistry resistant to proteases (Cara)
Synergy Pharmaceuticals Inc. (NY, USA)	Uroguanylin analogue	Dolcanatide **	Chemical modification
Emisphere Technologies, Inc. (NJ, USA) with Novo-Nordisk (Denmark)	Eligen®/ Novo insulin candidate	Insulin**	PE: N-acylated alpha-amino acid (undisclosed)
Diasome Pharma (OH, USA)	HDV-I	Insulin**	Liver-targeted liposomes
Sigmoid Pharma (Ireland)	SmPill®	CsA** for local Local colonic delivery	Oil in water emulsion

Enteris, Synergy, and Emisphere have other candidates further on in clinical development (Table 4). CsA: cyclosporine A.

Table 4. Oral peptide formulations in Phase III (completed/failed)

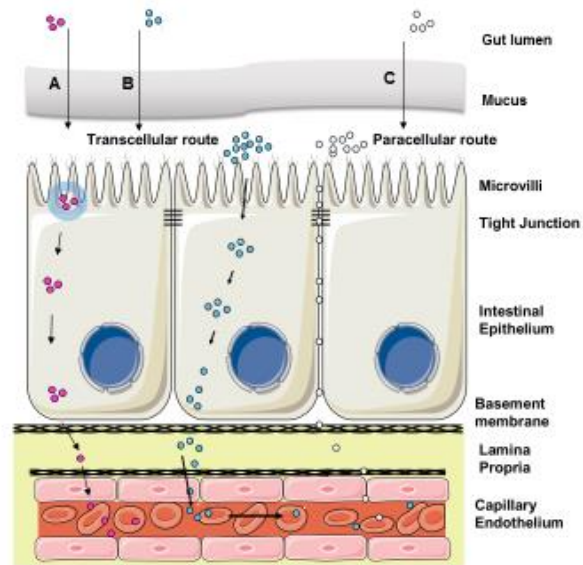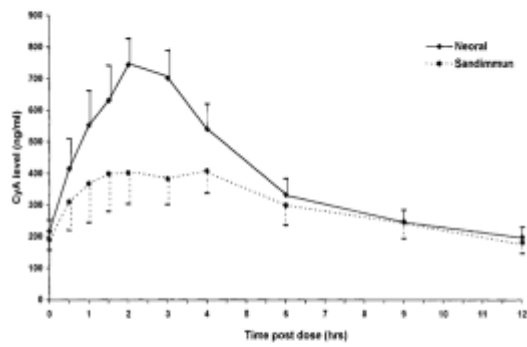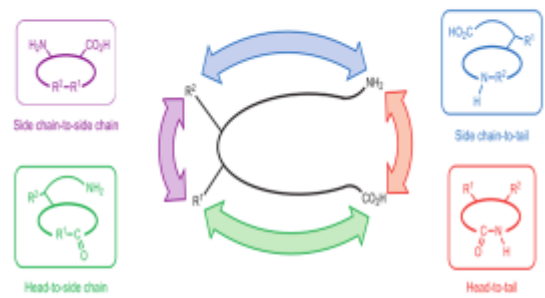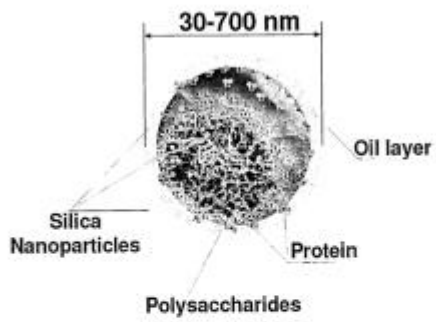| Company	Technology/Result	Peptide	Strategy
Tarsa therapeutics, Inc. (PA, USA)	Peptelligence™/ TBRIA ™ (met endpoints)	Salmon calcitonin NDA filed 2015	Local pH modulator: CA
Chiasma, Ltd. (Israel)	TPE®/ Mycapssa™ (met endpoints)	Octreotide NDA filed 2015	PE: sodium caprylate
Synergy Pharmaceuticals Inc. (NY, USA)	Uruguanylin analogue (met endpoints)	Plenacatide	Chemical modification. Local delivery
Biocon Ltd (India)	IN-105* (did not met endpoints)	Insulin-alkylated PEG prodrug insulin conjugates	Chemical modification
Emisphere Technologies, Inc. (NJ, USA) with Nordic Biosciences (Denmark) and Novartis (Switzerland)	Eligen®/ (Studies 2301 and 2302 did not met endpoints in osteoarthritis patients)	sCT (SMC021)	PE: 8-(N-2-hydroxy-5-chlorobenzoyl)-amino-caprylic acid (5-CNAC)
Emisphere Technologies, Inc. (NJ, USA) with Nordic Biosciences (Denmark) and Novartis (Switzerland)	Eligen®/ (Study 2303 did not met endpoints for osteoporosis patients)	sCT (SMC021)	PE: 5-CNAC
Emisphere Technologies, Inc. (NJ, USA) with Novo-Nordisk (Denmark)	Eligen®/ NN9924 / OG217SC (PIONEER Phase IIIa started 2015)	Semaglutide (long-acting GLP-1)	PE: Sodium N-[8-(2-hydroxybenzoyl) Amino] Caprylate (SNAC)

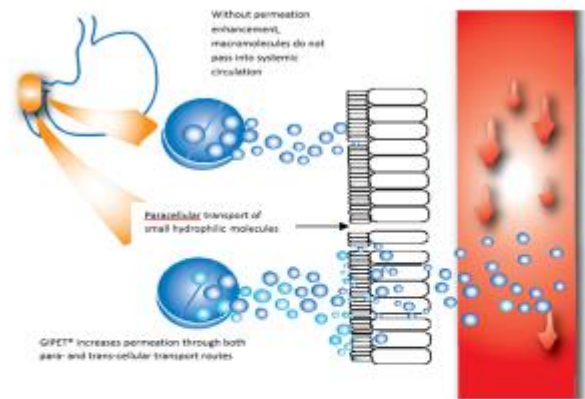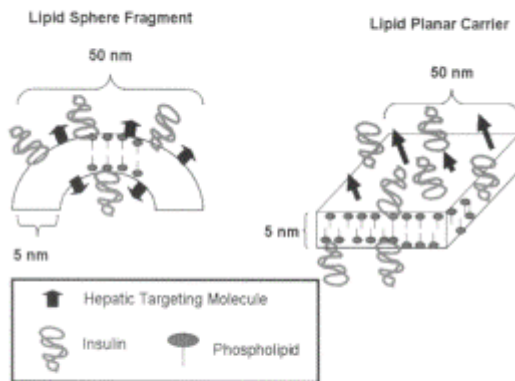* Started new Phase II trial recently.

Fig. 1

Fig. 2

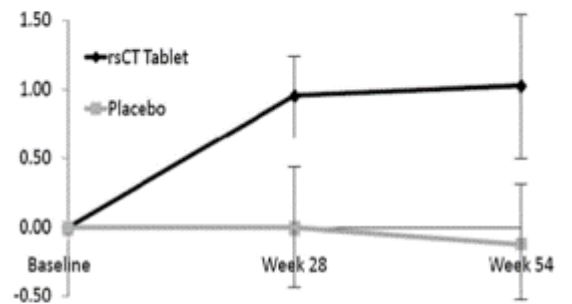
Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

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Fig. 8

Fig. 9

- Chemical modification (back in Phase II)
- Liposomes (Phase II)
- PE: aromatic alcohols (Phase II)
- PE: EDTA, bile salts + enzyme inhibitor: soy bean trypsin inhibitor, aprotinin (Phase II)
- PE: sodium caprate. (Phase II)
- Nanoparticles (Phase I)
- Bio-adhesive nanoparticles (Phase I)
- Micro-needles with citric acid (preclinical)
- Solid lipid nanoparticles (preclinical)

Insulin

- Local pH modulator: CA (Phase III, met endpoint)
- PE: N-acylated alpha-amino acid (5-CNAC) (Phase III, did not met endpoints)
- PE: aromatic alcohols (Phase II)
- Oil in water emulsion+ PE: bile salt, alkylsaccharides, sodium caprate (preclinical)

sCT

Uruguanylin analogue

- Chemical modification (Phase III, met endpoint)
- Chemical modification (Phase II)

Peptides in oral R & D

- Chemical modification (Phase III, met endpoint)
- PE: N-acylated alpha-amino acids (Phase III)
- PE: sodium caprate.(Phase II)
- PE: alkylsaccharides (preclinical)

GLP-1 analogue

PTH

- Chemical modification (Phase III, met endpoint)
- PE: acylcarnitine and citric acid (Phase II)
- PE: aromatic alcohols (Phase I)
- PE: alkylsaccharides (preclinical)

Octreotide

- PE: sodium caprylate (Phase III, met endpoint)
- PE: alkylsaccharides (preclinical)

Fig. 10

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