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The unprecedented number of marketing approvals in 2012 for peptide therapeutics could be a harbinger for the innovative peptide-based drugs in the clinical pipeline.

Future directions for peptide therapeutics development

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The notable expansion of peptide therapeutics development in the late 1990s and the 2000s led to an unprecedented number of marketing approvals in 2012 and has provided a robust pipeline that should deliver numerous approvals during the remainder of the 2010s. To document the current status of the pipeline, we collected data for peptide therapeutics in clinical studies and regulatory review, as well as those recently approved. In this Foundation review, we provide an overview of the pipeline, including therapeutic area and molecular targets, with a focus on glucagon-like peptide 1 receptor agonists. Areas for potential expansion, for example constrained peptides and peptide–drug conjugates, are profiled.

Introduction

The year 2012 proved to be a remarkable one for the peptide therapeutics sector. The expansion of the commercial clinical pipeline of these drugs during the late 1990s and 2000s ultimately led to first marketing approvals in 2012 for six peptides, the most ever to receive approvals as new molecular entities in a single year. All six peptides (i.e. lucinactant, peginesatide, pasireotide, carfilzomib, linaclotide, teduglutide) were approved in the USA; five of the six (peginesatide was the exception) were also approved in the European Union (EU). The near-term prospects for additional approvals are excellent because the pipeline includes more than a dozen molecules in late-stage clinical studies and marketing applications for two peptides (i.e. afamelanotide, albiglutide) are undergoing regulatory review. The year 2013, however, has seen mixed results so far. A first approval for one peptide (i.e. lixisenatide) has been granted, but one peptide (i.e. peginesatide) approved in 2012 was withdrawn because of safety issues.

Recent successes in the development of peptides as therapeutics, most notably glucagon-like peptide 1 receptor (GLP-1R) agonists such as liraglutide (Victoza®), are likely to spur additional growth in this sector. To track development trends for peptide therapeutics, we collected data from the public domain (e.g. company websites, clinicaltrials.gov, medical literature) for the peptides currently in clinical study sponsored by commercial firms. Specific data collected included descriptions of the peptides (e.g. amino acid sequence, modifications), target and development status (i.e. phase of clinical study, regulatory review, marketed). Synthetic peptides

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BOX 1

Data sources and definitions

Between 2008 and 2011 the Peptide Therapeutics Foundation (PTF) maintained and made publicly available a dataset of commercially sponsored protein therapeutics that had entered clinical study. The data collected for the analyses presented here expanded and updated the PTF's dataset, which was withdrawn by mid-2012. Data were collected from the public domain (e.g. clinicaltrials.gov, PubMed, company and regulatory agency websites). The information was cross-referenced with that available in commercial databases (e.g. Thomson Reuters Partnering™, Thomson Reuters Integrity℠, Sagient Research Systems BioMedTracker). The dataset of ~440 peptides was accurate to the best of our knowledge in mid-February 2013; however, it should be noted that some data (e.g. development status) change over time owing to the highly dynamic nature of the clinical pipeline.

For inclusion in the dataset, peptides were minimally defined as biologically active molecules composed of two amino acids coupled to each other through an amide or disulfide bond. Synthetic peptides of all lengths, including those conjugated to proteins, and recombinant peptides up to 50 amino acids were included; however, recombinant proteins, including insulin, were excluded. Molecules with peptide moieties that contribute to biologic activity but are not pharmacologically active (e.g. NGR-hTNF) were included. Semisynthetic peptides were evaluated for inclusion on a case-by-case basis but, as a class, peptides produced by fermentation were excluded. As defined by unique composition of matter, each peptide was included only once (i.e. data for various formulations, doses, routes of administration and stages of development were embedded in a single record). Development status was assigned based on the most advanced clinical study and each peptide was assigned a single therapeutic area based on the indication of the most advanced clinical study.

of any length and recombinant peptides up to 50 amino acids were included; modified peptides (e.g. linked to albumin, immunoglobulin) and semisynthetic peptides were considered for inclusion on a case-by-case basis (Box 1 presents data sources and inclusion and/or exclusion criteria). The data supplemented and updated a dataset maintained by the Peptide Therapeutics Foundation of commercially sponsored peptide therapeutics that have been evaluated in clinical study.

Here, we profile recently approved products and those that could be approved soon, and provide an overview of the pipeline that focuses on peptides undergoing evaluation in studies that could be completed in 2013 and those that entered studies recently. The current emphasis on development of peptides with enhanced properties (e.g. extended half-life, dual agonist activity) and the possibilities for innovative modalities such as peptide–drug conjugates and constrained peptides as therapeutics are also examined. Owing to the large volume of literature for the peptides described here, only selected references are provided.

Peptide therapeutics recently approved and in review

The USA and EU are the major markets for drugs of all kinds and, as a consequence, first approvals for peptide therapeutics have occurred primarily in one of these two regions. All of the 19 peptides approved in the USA during the period 2001 to 2012 were first approved in either the USA or EU. The rate of US approvals for peptide therapeutics substantially increased after the large number of approvals in 2012; the rate rose from 1.3 per year in the 2000s to three per year for the first three years of the 2010s. On average, the clinical development and US approval phase lengths for the six peptides approved in 2012, all of which received a standard review, were nine and 2.1 years, respectively. The median phase lengths were, however, notably shorter (7.8 and 0.95 years, respectively) compared with the averages.

The peptides approved in 2012 (Table 1) are notable for their number and the diversity of their uses. Two (i.e. linaclotide, teduglutide) are for gastrointestinal disorders, one (i.e. lucinactant) is a lung surfactant used to prevent respiratory distress syndrome in high-risk, premature infants, one (i.e. peginesatide) is a treatment for anemia in adult dialysis patients who have chronic kidney disease, one (i.e. pasireotide) is used to treat adult patients with Cushing's disease for whom pituitary surgery is not an option or has not been curative and one (i.e. carfilzomib) is a treatment for a hematological cancer. Although peptides are commonly thought of as intravenously (i.v.) administered drugs, only one of the six peptides (i.e. carfilzomib) is solely i.v. administered. Three (i.e. peginesatide, pasireotide, teduglutide) can be administered subcutaneously (s.c.), one is inhaled (lucinactant) and one (i.e. linaclotide) is orally delivered.

TABLE 1

Peptide therapeutics gaining first marketing approvals during 2012

Company	Brand name	INN	Mechanism of action	Region (date) approved	Approved indication
Discovery Laboratories	Surfaxin®	Lucinactant	Surfactant	USA (6 March 2012)	RDS in premature infants
Affymax	Omontys®	Peginesatide	EPO receptor agonist	USA (27 March 2012); Regulatory review in EU	Anemia
Novartis	Signifor®	Pasireotide	Somatostatin receptor agonist	EU (24 April 2012); USA (14 December 2012)	Cushing's disease
Onyx Pharmaceuticals	Kyprolis®	Carfilzomib	Chymotrypsin-like protease inhibitor	USA (20 July 2012)	Multiple myeloma
Ironwood Pharmaceuticals/ Forest Laboratories, Almirall	Linzess® (USA); Constella® (EU)	Linaclotide	GC-C receptor agonist	USA (30 August 2012); EU (26 November 2012)	Constipation-predominant IBS, chronic idiopathic constipation
NPS Pharmaceuticals	Revestive® (EU); Gattex® (USA)	Teduglutide	GLP-2 receptor agonist	EU (30 August 2012); USA (21 December 2012)	Short bowel syndrome

Data current as of 15 February 2013. *Abbreviations*: EPO: erythropoietin; GC-C: guanylate cyclase-C; GLP: glucagon-like peptide; NOD2: nucleotide-binding oligomerization domain-containing protein 2; RDS: respiratory distress syndrome.

Although the six approvals in 2012 are a significant feat for the industry, the voluntary recall less than a year after approval of one (i.e. peginesatide) demonstrates the risk and unpredictability of drug development. The recall is a result of post-approval reports of hypersensitivity reactions occurring in patients soon after their first i.v. dose; three cases resulted in death. The recall includes i.v. and s.c. formulations of peginesatide. This safety issue was not identified during the clinical trials of the drug. The FDA medical review of peginesatide in fact indicates that hypersensitivity or anaphylactic-type reactions were not reported among over 2300 patients who were administered one or more doses of the drug [1]. At the time of this writing, it is too early to know how or if peginesatide could be used again in the clinic, but certainly this outcome highlights the risks of drug development and the power of post-approval surveillance in identifying safety issues.

As of February, one peptide therapeutic had already been approved in 2013. Lixisenatide (Lyxumia®; Sanofi), a GLP-1R agonist, was approved in the EU as a treatment for type 2 diabetes; the product is undergoing regulatory review in the USA. Lixisenatide will compete with two other GLP-1R agonist peptides, exenatide (Byetta®, Bydureon®; Bristol-Myers Squibb) and liraglutide (Victoza®; Novo Nordisk), already marketed in the EU and USA for type 2 diabetes. Exenatide was first approved in 2005 in the USA, whereas liraglutide was first approved in 2009 in the EU. The three products are all administered s.c. but they differ in their frequency of administration. The formulation of exenatide marketed as Byetta® is administered twice daily. Bydureon®, which is an extended-release formulation of exenatide first approved in the USA in 2012, is administered once weekly. Liraglutide and lixisenatide are administered once daily.

Two peptide therapeutics not yet approved in any country are undergoing regulatory review as of February 2013. Albiglutide (GlaxoSmithKline), a GLP-1R agonist peptide fused to human serum albumin (HSA), is in regulatory review in the EU as a type 2 diabetes treatment with once weekly dosing. The photoprotectant afamelanotide (Clinuvel Pharmaceuticals), a melanocortin 1 receptor agonist, is undergoing review in the EU for erythropoietic protoporphyria (EPP), a rare genetic disease characterized by severe reactions to sunlight. Afamelanotide, which is delivered via a dissolvable s.c. implant given once every two months, has orphan designations in the EU and USA for the treatment of EPP and solar urticaria.

Peptide therapeutics pipeline

The clinical pipeline is currently composed of 128 peptide therapeutics, with 40 in Phase I studies, 74 that have advanced to Phase I/II or Phase II studies and 14 that are in Phase II/III or Phase III studies. The peptides at each phase of development are undergoing evaluation for a wide variety of indications (Fig. 1). The diversity of therapeutic areas (TAs) represented is substantially higher in Phase I and Phase II compared with Phase III, which is at least in part a consequence of the greater number of peptides in early-stage compared with pivotal studies. Taken together, the top two TAs comprise a minority of the peptides in Phase I and Phase II studies (40% and 29%, respectively) but a majority (i.e. 57%) of those in Phase III studies. The top two TAs are metabolic disease and oncology for the Phase I and II peptides, whereas oncology and infectious disease are the top two TAs for peptides in Phase III.

Peptides to watch in 2013

Entry into pivotal studies intended to provide safety and efficacy data to support a marketing application represents a milestone in the development of a drug. Of the 14 peptides currently in Phase III studies (Table 2), four (i.e. NGR-hTNF, oritavancin, dulaglutide,

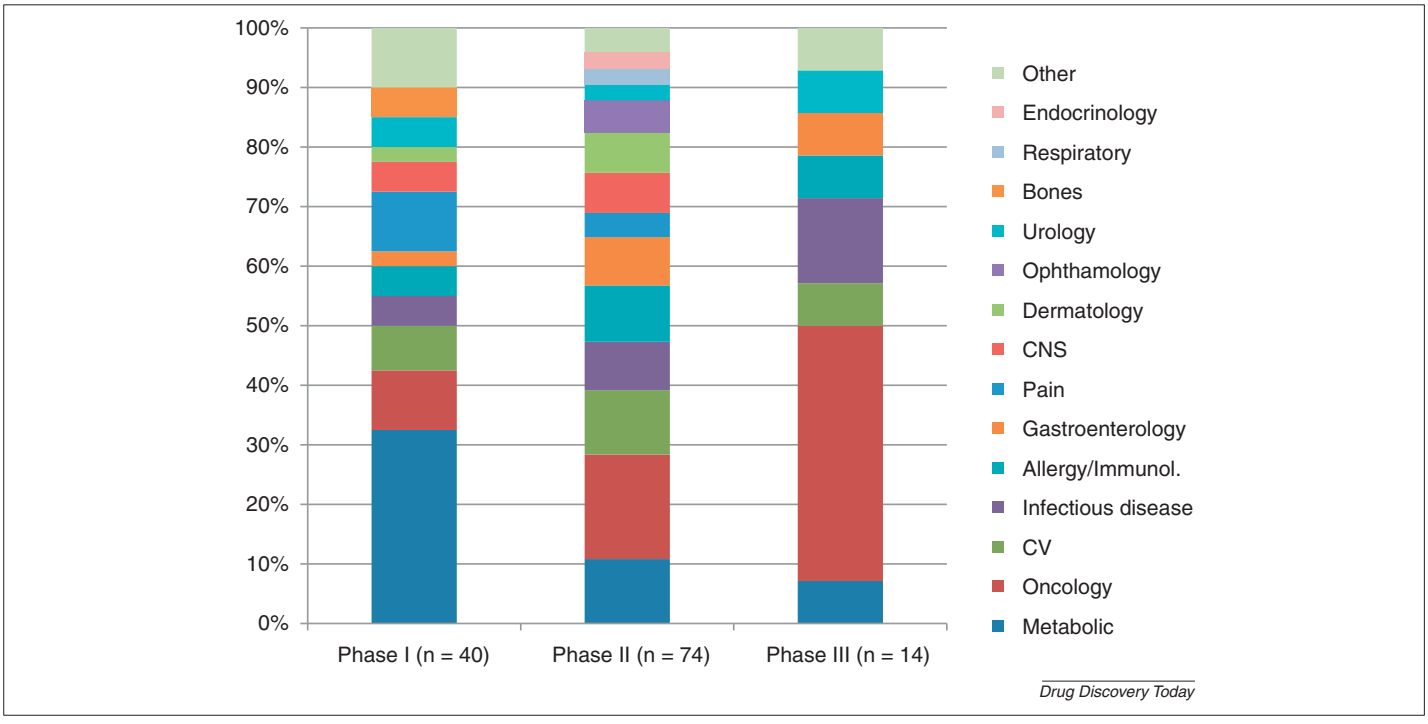

FIGURE 1

Therapeutic areas for peptides in clinical studies. Data current on 15 February 2013. *Abbreviations*: CNS: central nervous system; CV: cardiovascular.

TABLE 2

Peptide therapeutics in Phase III clinical studies

Company sponsoring clinical study	INN or code name	Mechanism of action	Indication of Phase III studies
Merck Serono	Cilengitide	αvβ3, αvβ5 antagonist	Glioblastoma
MolMed	NGR-hTNF	Aminopeptidase N antagonist	Mesothelioma
Amgen	Trebananib	Ang-1, Ang-2 antagonist	Ovarian, peritoneal or fallopian tube cancers
Advanced Accelerator Applications	177Lu-DOTA0-Tyr3-Octreotate	Somatostatin receptors	Midgut carcinoid tumors
China Medical System/Kangzhe Pharmaceutical (Shenzhen)	Tyroserleutide	Tumor inhibitor with multiple MOA	Hepatocellular carcinoma
The Medicines Company	Oritavancin	Gram-positive bacterial cell membrane disruption	Acute bacterial skin and skin structure infections
Cubist Pharmaceuticals	CB183315	*C. difficile* cell membrane disruption	*Clostridium-difficile*-associated diarrhea
Eli Lilly	Dulaglutide	GLP-1R agonist	Type 2 diabetes mellitus
Novo Nordisk	Semaglutide	GLP-1R agonist	Type 2 diabetes mellitus
Cardiorentis	Ularitide	NPR-A agonist	Acute decompensated heart failure
Anthera Pharmaceuticals	Blisibimod	B-cell-activating factor inhibitor	Systemic lupus erythematosus
Radius Health	BA058	PTHR agonist	Osteoporosis
Nymox Pharmaceutical	NX1207	Proapoptotic	Benign prostatic hyperplasia
Derma Sciences	DSC127	Angiotensin analog with multiple MOA	Chronic, nonhealing diabetic foot ulcers

Data current on 25 April 2013. *Abbreviations*: GC-C: guanylate cyclase-C; GLP-1R: glucagon-like peptide 1 receptor; INN: international non-proprietary name; NPR-A: atrial natriuretic peptide receptor; MOA: mechanism of action; NA: not available; PTHR: parathyroid hormone-related peptide receptor.

BA058 and NX1207) are in studies with estimated completion dates in 2013. The results of these studies could determine whether the molecules proceed in development or to regulatory review. NGR-hTNF (MolMed) is composed of a tumor-homing peptide (Asn-Gly-Arg; NGR) [2] conjugated to human tumor necrosis factor (hTNF) [3]. Low (0.8 $\mu g/m^2$) weekly doses of NGR-hTNF combined with best investigator's choice (BIC) of single-agent chemotherapy (i.e. doxorubicin, gemcitabine or vinorelbine) are being compared with placebo combined with BIC in a Phase III study (i.e. NCT01098266) of previously treated patients with advanced malignant pleural mesothelioma. The estimated study completion date is February 2013. NGR-hTNF in combination therapy is also being evaluated in Phase II studies that are recruiting patients with non-small-cell lung cancer, soft tissue sarcomas and ovarian cancer as of February 2013.

Oritavancin (The Medicines Company), a semisynthetic lipoglycopeptide antibiotic [4], is undergoing evaluation in two Phase III studies of identical design, SOLO-1 (i.e. NCT01252719) and SOLO-2 (i.e. NCT01252732). Both Phase III studies are designed to assess the efficacy, safety and tolerability of oritavancin vs vancomycin as a treatment for acute bacterial skin and skin structure infections (ABSSSI) caused by susceptible gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). Top-line results for the SOLO-1 study announced by The Medicines Company in December 2012 indicated that oritavancin was non-inferior to vancomycin in the efficacy analyses at endpoints required by US and EU regulators, and safety profiles for the two drugs were similar. The SOLO-2 study has an estimated completion date of June 2013.

The GLP-1R agonist dulaglutide (Eli Lilly) has been or is being evaluated in at least 14 Phase II/III or Phase III studies in patients with type 2 diabetes, including the eight AWARD studies. In the AWARD study series, dulaglutide administered s.c. weekly is being compared to other diabetes drugs (i.e. exenatide, insulin glargine, metformin, sitagliptin, liraglutide) or placebo in type 2 diabetes patients. The AWARD-1 through to AWARD-5 studies were completed in 2012; AWARD-6, -7 and -8 are due for completion during 2014–2015. Study sites located in Japan are recruiting type 2 diabetes patients for two ongoing Phase III studies (i.e. NCT01468181, NCT01584232) of dulaglutide; these studies have estimated completion dates of July and December 2013, respectively.

A Phase III study (i.e. NCT01343004) is evaluating a s.c. formulation of BA058 (Radius Health), an analog of human-parathyroid-hormone-related protein, for the prevention of fracture in post-menopausal women with severe osteoporosis who are ambulatory and at risk of fracture. The effects of BA058 and teriparatide administered s.c. daily are being compared in this study, which has an estimated completion date of December 2013. NX1207 (Nymox), a proapoptotic peptide [5], is undergoing evaluation in three Phase III studies in patients with benign prostatic hyperplasia. The safety and efficacy of a second transrectal intraprostatic injection of NX1207 given to patients who previously received an injection in an earlier US study of the drug were assessed in a Phase III trial (i.e. NCT01438775) with an estimated completion date of January 2013. Two additional Phase III studies (i.e. NCT00918983, NCT00945490) are due for completion in November 2013.

Peptides in proof-of-concept studies

The greatest diversity in the pipeline is found within the peptide therapeutics at Phase II, a group that includes 74 peptides (11 in Phase I/II and 63 in Phase II studies). A variety of molecular formats (e.g. peptides linked to small molecules, lipids, carbohydrates, biopolymers, polyethylene glycol or proteins) and mechanisms of action (e.g. cell-targeting peptides, cell-penetrating peptides) are represented in this group, and they are being evaluated as treatments for diseases in more than 13 therapeutic areas.

Nine peptides (12%) in Phase II studies were designed to incorporate moieties that increased the functionality of the molecule. Of these, two peptides (i.e. zoptarelin doxorubicin, EP100) in particular exemplify the molecular diversity possible using peptides. Both molecules are cell-targeting peptides composed of a luteinizing-hormone-releasing hormone (LHRH) receptor-targeting ligand linked to a cytotoxin, and thus are classified as peptide–drug conjugates (PDCs) – both are being studied as cancer drugs. Zoptarelin doxorubicin (Aeterna Zentaris), also known as AEZS108 or AN152, comprises a peptide LHRH analog coupled to the small molecule cancer drug doxorubicin. In the case of EP100 (Esperance Pharmaceuticals), the cytotoxic moiety is the lytic peptide CLIP71, which disrupts cell membranes. Zoptarelin doxorubicin is being studied as a treatment for a variety of LHRH receptor-positive tumors, including urothelial carcinoma, and prostate, breast and ovarian cancers [6]. A Phase III study (i.e. NCT01767155) comparing zoptarelin doxorubicin to doxorubicin as second-line therapy in endometrial cancer patients was not open for participant recruitment at the time of writing. EP100 in combination with paclitaxel is being compared to paclitaxel alone in a Phase II study (i.e. NCT01485848) of patients with refractory or recurrent ovarian cancer. The estimated completion date for the study is October 2013.

Two peptides (i.e. G202, PRX302) in Phase II studies are prodrugs. In the case of G202 (GenSpera) a thapsigargin prodrug is hyrdrolyzed by prostate-specific membrane antigen, which is a carboxypeptidase expressed on tumor endothelial cells [7], thereby releasing a drug at the tumor site [8]. A Phase II trial (i.e. NCT01777594) of G202 as second-line therapy in hepatocellular carcinoma patients was initiated in January 2013, and a Phase II study (i.e. NCT01734681) in patients with castrate-resistant prostate cancer is due to start in May 2013. PRX302 (Sophiris Bio) is a modified form of proaerolysin designed to be cleavable by enzymatically active prostate-specific antigen (PSA), a member of the kallikrein family of proteases found in prostate tissue [7]. Production of the pore-forming, cytolytic aerolysin is thus localized to cells expressing PSA [9]. A Phase I/II study (i.e. NCT01454349) of PRX302 as a treatment for lower urinary tract symptoms secondary to benign prostatic hyperplasia is due for completion in February 2013.

Half-life extension was the rationale for conjugation of four peptides (i.e. CBX129801, CVX060, LAPSExd4, PB1023) in Phase II studies. Peptides typically have short half-lives (i.e. minutes) in circulation, which limits therapeutic utility. Half-life, however, can be substantially extended (i.e. to days or weeks) through attachment of the peptide to molecules such as polyethylene glycol (PEG) or immunoglobulin G (IgG). CBX129801 (Cebix) is a PEGylated formulation of human C-peptide that can be administered s.c. once weekly; a Phase II study (i.e. NCT01681290) in type 1 diabetes patients with mild-to-moderate diabetic peripheral neuropathy was initiated in October 2012. CVX060 (Pfizer) and LAPSExd4 (Hanmi Pharmaceutical) are composed of peptides conjugated to IgG or an IgG fragment, and they are administered weekly.

The design of drugs that can predictably penetrate the cell membrane and target intracellular processes is of intense interest to the pharmaceutical industry. The cellular membrane acts as an effective barrier to the entry of many drugs, including peptides, and, as a consequence, few peptides currently in the pipeline have intracellular targets. Peptides that do enter cells are typically cationic or amphipathic molecules, including some that incorporate so-called cell-penetrating peptide (CPP) sequences (also known as protein transduction domains) such as the transcription transactivating (TAT) sequence of the human immunodeficiency virus [10]. Of the peptides in Phase II studies, three (i.e. CBP501, AM111, ACT1) are CPPs. Composed of residues 211–221 of CDC25C connected to TAT, CBP501 (CanBas) interferes with phosphorylation of CDC25C and abrogates G2 arrest caused by DNA-damaging agents [11,12]. A Phase I/II study (i.e. NCT00700336) of a combination of CBP501, pemetrexed and cisplatin in two cohorts, patients with advanced solid tumors and chemotherapy-naive, malignant pleural mesothelioma patients, was completed in November 2012. A Phase II trial (i.e. NCT00942825) of the same drug combination as first-line treatment in non-small-cell lung cancer patients is ongoing, but not recruiting patients. AM111 (Auris Medical), also known as XG102 and DJNKI1, includes a JNK-binding domain and a ten amino acid TAT sequence [13]. The peptide has been evaluated in clinical studies as a treatment for hearing loss. In a preliminary Phase I/II study of 11 patients with acute acoustic trauma, intratympanic treatment appeared to have a therapeutic effect; quantification of the benefit was not possible because the study did not include a control group [14]. A Phase II study (i.e. NCT00802425) of AM111 as a treatment for acute inner-ear hearing loss was completed in October 2012. Instead of TAT, ACT1 (FirstString Research) exploits a 16 amino acid cationic internalization sequence from Antennapedia, a *Drosophila* homeoprotein, for delivery of the C-terminus of connexin 43 into cells [15]. Three Phase II studies to evaluate the safety and efficacies of Act1 for scar reduction of acute surgical wounds and the treatment of chronic wounds, diabetic foot ulcers and venous leg ulcers were conducted in India.

Peptides in Phase I studies

Signaling the substantial interest in peptide therapeutics development by the biopharmaceutical industry, our data show that more than half (i.e. 23 out of 40; 58%) of the peptides currently in Phase I studies entered this phase of development within the past two years. Compared with those in Phase II, a larger percentage of peptides in Phase I are conjugated to another moiety (12% vs 20%, respectively). Another distinguishing feature of the peptides in Phase I is the focus on metabolic disorders. Nearly one-third of peptides in Phase I are intended to be treatments for metabolic disorders (compared with only 11% and 8% of those at Phase II and Phase III, respectively), and most (85%) of these were either mono or dual agonists of GLP-1R. This intense focus on GLP-1R as a target reflects the recognition of the opportunities for development of innovative peptides as diabetes treatments, and two peptides (i.e. RO6811135, ZP2929) in Phase I exemplify a new class of such drugs (i.e. dual agonists). RO6811135 (Hoffmann-La Roche) is a dual agonist of GLP-1R and the gastric inhibitory polypeptide (GIP) receptor. A Phase I study (i.e. NCT01676584) to investigate the safety, tolerability, pharmacokinetics and pharmacodynamics of RO6811135 in healthy subjects was completed in January 2013. ZP2929 (Boehringer Ingelheim/Zealand Pharmaceuticals) and TT401 (Transition Therapeutics) are dual agonists of GLP-1R and the glucagon receptor. ZP2929 is being developed as a treatment for diabetes and obesity; it is under assessment in a Phase I

study of its safety and tolerability in healthy volunteers. The safety, tolerability and pharmacokinetics of single ascending doses of TT401 were evaluated in a Phase I study of healthy obese volunteers. A study of TT401 in obese type 2 diabetes patients is planned.

ALRN5281 (Aileron Therapeutics) is the first in a new class of constrained peptides to enter the clinical pipeline. The stapling process at Aileron constrains the flexibility of molecules. The safety and tolerability of s.c. administration of ALRN5281, a growth-hormone-releasing hormone agonist that can potentially treat metabolic disorders, is being evaluated in a Phase I study (i.e. NCT01775358) of healthy volunteers that was initiated in January 2013.

Target diversity in the peptide pipeline

The diversity of the indications for which peptide therapeutics are studied results directly from the diversity of their targets. Quantitative analysis of the target diversity for peptides in clinical study was complicated because some of the drugs have complex or poorly understood mechanisms of action, or act on multiple targets. Nevertheless, the available data indicate that more than half of the peptides in clinical study have unique targets (i.e. only one peptide in the pipeline was known to act on the particular target). Approximately 10% of the pipeline peptides targeted bacterial, viral or fungal organisms. As groups, peptides targeting ion channels, non-transducing receptors, pumps and transporters, receptor enzymes, nonenzymatic transmembrane proteins or intracellular targets of all kinds each comprised <10% of the total pipeline.

The largest target group was composed of the cell surface molecules, in particular the G-protein-coupled receptors (GPCRs). This result is not surprising because the accessibility and diverse functions of GPCRs have made them attractive targets for drugs of any composition [16]. Overall, 39% of peptides in the clinical pipeline target GPCRs, with approximately one-third of these targeting the GLP-1 and GLP-2 receptors, and the remainder targeting a wide variety of other GPCR targets (Fig. 2).

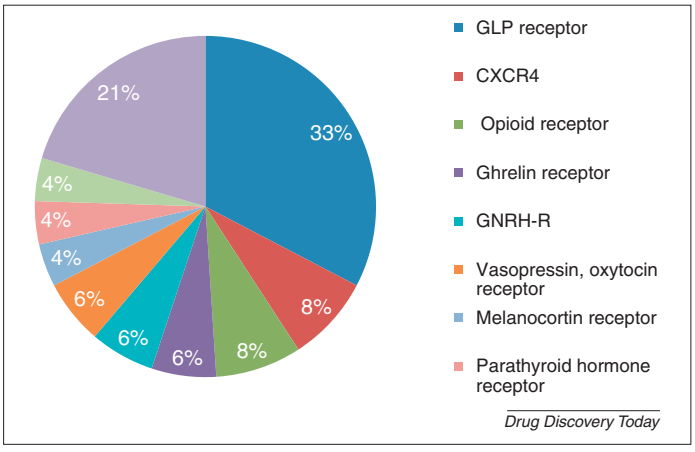

FIGURE 2

G-protein-coupled receptor targets of peptides in clinical studies. GPCR targets for two or more peptides in the pipeline are identified by name; GPCR targets for just one peptide are represented as a single cohort. Data current as of 15 February 2013. *Abbreviations*: CXCR: chemokine receptor type 4; GLP: glucagon-like peptide; GNRH-R: gonadotropin-releasing hormone receptor; GPCR: G-protein-coupled receptor.

GLP-1R agonists

The single most frequent target for peptide therapeutics evaluated in clinical studies is GLP-1R. Of 265 peptide therapeutics that entered clinical study during 2000–2012, 32 (12.1%) were GLP-1R agonists; all other targets were below 3% in frequency. The receptor is well-validated as a target for type 2 diabetes drugs [17,18] and, for the most part, GLP-1R agonists have been studied as treatments for diabetes. Peptide therapeutics are natural choices for development compared with other modalities (e.g. antibodies, small molecules) because the endogenous ligand of GLP-1R, GLP-1(7–36)amide, is a peptide agonist. As of January 2013, three GLP-1R agonists were approved for marketing (i.e. exenatide, liraglutide, lixisenatide), one was at regulatory review (i.e. albiglutide) and 13 were undergoing evaluation in clinical studies (Table 3).

Since the approval of exenatide in 2005, a notable trend in the product category has been the development of peptides designed, formulated or delivered such that they can be dosed less frequently than the twice-daily regimen of exenatide. Binding of GLP-1 to its receptor causes numerous physiological effects (e.g. alteration of insulin and glucagon levels relevant to the treatment of diabetes and obesity) but the endogenous ligand is degraded within 1–2 min by dipeptidyl peptidase 4 (DPP4) [19]. Exenatide, which has a half-life of ~2.4 hours [20], and lixisenatide were specifically designed to be DDP4-resistant. Although it has a similar half-life (2–4 hours) [21], lixisenatide can be dosed once daily [22] owing to its high affinity for GLP-1R [$IC_{50} = 1.4 \pm 0.2$ nM compared with 5.5 ± 1.3 nM for GLP-1(7–36)amide] [23]. The peptide backbone of liraglutide was modified by addition of a lipid (i.e. palmitic acid). With a half-life of 13 hours [24], liraglutide can be administered once daily. The prolonged action profile of the product derives from slow absorption caused by self-association, binding to HSA and resistance to DDP4 degradation [25].

Development of GLP-1R agonists that can be dosed weekly, which can improve patient convenience and compliance, has been achieved using several methods. Albiglutide and dulaglutide comprise GLP-1R agonist peptides genetically fused to HSA or IgG. The protein moiety enables binding to the neonatal Fc receptor (FcRn) and subsequent protection from intracellular degradation [26,27]. In their endogenous forms, HSA and IgG have long half-lives (~19 days and ~26 days, respectively) as a result of FcRn-mediated recycling of the proteins. Albiglutide comprises a tandem repeat of a DDP4-resistant GLP-1(7–36)amide analog fused to HSA; the molecule has a half-life of 6–7 days [28]. Dulaglutide comprises a DDP4-resistant GLP-1(7–36)amide analog fused to the Fc region of an IgG4 that was engineered to reduce binding to Fcγ receptors and the potential for immunogenicity, and eliminate half-antibody formation [29]; it has a half-life of around four days [30]. In January 2013 GlaxoSmithKline announced that a marketing application for albiglutide as a treatment for type 2 diabetes in adults had been submitted to the FDA; as of February 2013, dulaglutide was undergoing evaluation in numerous ongoing Phase III studies of patients with type 2 diabetes.

Weekly administration of the GLP-1R agonists semaglutide (Novo Nordisk) and PB1023 (PhaseBio Pharmaceuticals) to patients with type 2 diabetes has been evaluated in Phase II studies. Semaglutide is an acylated GLP-1 analog with a half-life of 6–7 days [31,32]. Initiated in early 2013, the Phase III SUSTAIN™ 6 study (i.e. NCT01720446) is evaluating the

TABLE 3

Glucagon-like peptide-1 receptor agonists in clinical studies

Company sponsoring clinical study	INN (brand name) or drug code	Molecular type	Dosing	Status	Indication of clinical studies
Bristol-Myers Squibb	Exenatide (Byetta®; Bydureon®)	39 aa peptidase-resistant peptide	s.c. twice daily (Byetta®) or once weekly (Bydureon®)	Approved for T2 diabetes	T2 diabetes, obesity, CV
Novo Nordisk	Liraglutide (Victoza®)	31 aa peptide linked to lipid	s.c. once daily	Approved for T2 diabetes	T2 diabetes, obesity, CV
Sanofi	Lixisenatide (Lyxumia®)	44 aa peptidase-resistant peptide	s.c. once daily	Approved for T2 diabetes	T2 diabetes, CV
GlaxoSmithKline	Albiglutide	Tandem repeat of 30 aa peptide fused with human albumin	s.c. once weekly	Regulatory review	T2 diabetes
Eli Lilly	Dulaglutide	46 aa peptide fused with IgG4 Fc	s.c. once weekly	Phase III	T2 diabetes
Novo Nordisk	Semaglutide	37 aa acylated peptide	s.c. once weekly	Phase III	T2 diabetes
PhaseBio Pharmaceuticals	PB1023	Peptide fused with ELP biopolymer	s.c. once weekly	Phase II	T2 diabetes
Hanmi Pharmaceutical	HM11260C, LAPS-Exendin	Exendin-4 analog conjugated to human Ig fragment	s.c. once weekly or once monthly	Phase II	T2 diabetes
Diartis Pharmaceuticals	Exenatide-XTEN, VRS859	Exenatide linked to hydrophilic amino acids	s.c. once monthly	Phase I	T2 diabetes
Pfizer	CVX096	Peptide conjugated to IgG	s.c.	Phase I	T2 diabetes
GlaxoSmithKline	GSK2374697	GLP-1R agonist, domain antibody	s.c.	Phase I	T2 diabetes
Novo Nordisk	NN9924, OG217SC	31 aa peptide; long-acting	Oral	Phase I	T2 diabetes
Novo Nordisk	NN9926, OG987GT	GLP-1 analog; long-acting	Oral	Phase I	T2 diabetes
Zydus-Cadila Group	ZYOG1	GLP-1 agonist	Oral	Phase I	T2 diabetes
Hoffmann-La Roche	RO6811135, MAR709	16 aa pegylated peptide; dual agonist	s.c.	Phase I	T2 diabetes
Transition Therapeutics	TT401	Dual agonist	s.c. once weekly	Phase I	T2 diabetes, obesity
Zealand Pharma	ZP2929	Dual agonist	s.c. once daily	Phase I	T2 diabetes, obesity

Data current on 15 February 2013. *Abbreviations*: aa: amino acid; CV: cardiovascular effects; ELP: elastin-like peptide; INN: international non-proprietary name; NA: not available; SC: subcutaneous; T2 diabetes: type 2 diabetes mellitus.

cardiovascular and other long-term outcomes of weekly administration of semaglutide to patients with type 2 diabetes. PB1023 is a recombinant GLP-1 analog fused to a proprietary biopolymer; a Phase IIb study (i.e. NCT01658501) to evaluate once-weekly dosing in adults with inadequately treated type 2 diabetes was ongoing as of February 2013. Patient convenience and compliance with dosing regimens might also be improved by the development of GLP-1R agonists that can be dosed monthly or those that can be delivered orally. Several such product candidates are in early clinical studies (Table 3).

Since exenatide was approved in 2005, long-acting GLP-1R agonists that benefit patients have been developed, but the evolution of this class of peptides is not complete. Improved products could result from the development of dual agonists that target GLP-1R as well as another receptor relevant in diabetes [33]. As we previously noted, three dual agonists, one targeting GLP-1R and the GIP receptor (i.e. RO6811135) and two targeting GLP-1R and the glucagon receptor (i.e. ZP2929, TT401), have entered Phase I within the past two years.

Beyond type 2 diabetes

Because of the substantial resources dedicated to the development of GLP-1R agonists for type 2 diabetes, knowledge of the full range of physiological responses that result from interactions of these drugs with GLP-1R has substantially increased over the past decade. Potential applications for GLP-1R agonists in weight management, cardiovascular diseases and neurodegenerative disorders are being explored in clinical studies sponsored by commercial and noncommercial organizations. Marketing approvals of the products for treatment, or prevention, of conditions such as obesity, myocardial infarction or Alzheimer's disease could substantially expand the markets for GLP-1R agonists in the future. Relatively few studies, however, have been completed in these alternate indications.

Liraglutide was evaluated in a Phase II study (i.e. NCT00422058) of body weight loss in obese, nondiabetic subjects. Treatment over 20 weeks was well-tolerated, induced significant weight loss compared with placebo and reduced blood pressure and pre-diabetes prevalence [34]. In an extension study (i.e. NCT00480909) weight loss was sustained over two years and cardiovascular risk factors were improved [35]. Study results are not yet available for the completed Phase III SCALE™ – maintenance study (i.e. NCT00781937) in which the effect of liraglutide on long-term weight maintenance and additional weight loss induced by a four to 12 week low calorie diet on obese, nondiabetic subjects was evaluated. Liraglutide is also undergoing evaluation in the ongoing Phase III SCALE™ – sleep apnea study (i.e. NCT01557166) of obese, nondiabetic subjects with moderate or severe obstructive sleep apnea and the Phase III

SCALE™ – obesity and pre-diabetes study (i.e. NCT01272219) of obese, nondiabetic subjects. These two studies are due for completion in June 2013 and February 2015, respectively. The effects of exenatide on energy expenditure and weight loss in nondiabetic obese subjects are being evaluated in a Phase III study (i.e. NCT00856609) sponsored by the National Institute of Diabetes and Digestive and Kidney Diseases. The estimated study completion date is February 2013.

The potential beneficial cardiovascular effects of GLP-1R agonists have recently been reviewed [36–38]. The extents to which exenatide, liraglutide, lixisenatide and semaglutide exert effects on cardiovascular outcomes such as cardiovascular-related death, nonfatal myocardial infarction or nonfatal stroke are being evaluated in clinical studies. The Phase III EXSCEL study (i.e. NCT01144338) is comparing standard care with and without exenatide on major cardiovascular outcomes in type 2 diabetes patients. The estimated completion date is March 2017. The Phase III LEADER® study (i.e. NCT01179048) is a long-term trial to determine the effects of liraglutide on cardiovascular events in type 2 diabetes patients; the estimated completion date is January 2016. The Phase III ELIXA study (i.e. NCT01147250) is evaluating whether lixisenatide can reduce cardiovascular morbidity and mortality compared with placebo in type 2 diabetes patients who recently experienced an acute coronary syndrome event. The estimated study completion date is May 2014. The Phase III SUSTAIN™ 6 study (i.e. NCT01720446) of semaglutide is evaluating cardiovascular and other long-term outcomes with semaglutide in type 2 diabetes patients.

Potential roles for GLP-1R agonists as treatments for neurodegenerative disorders and cognitive deficits have been elucidated [39,40], but clinical study in this area has so far been performed primarily by noncommercial sponsors. The National Institute on Aging is sponsoring an ongoing Phase II study (i.e. NCT01255163) comparing exenatide with placebo to determine long-term treatment outcomes for patients with early-stage Alzheimer's disease or mild cognitive impairment. University College London, UK, is sponsoring a Phase II study (i.e. NCT01174810) evaluating exenatide as a treatment for moderate severity Parkinson's disease. A clinical study (i.e. NCT01469351) sponsored by the University of Aarhus, Denmark, is assessing the effects of liraglutide on degenerative changes in Alzheimer's disease patients. In collaboration with Novo Nordisk, the University of Lübeck, Germany, is sponsoring a Phase I study (i.e. NCT01550653) of the effects of liraglutide on memory in healthy subjects. Both of these studies have estimated completion dates of June 2013.

Current and future commercial impact

To assess the commercial value of peptide therapeutics we analyzed annual sales data for 25 US-approved products, which together had global sales of US$14.7 billion in 2011. Seven products had global sales over US$500 million. With one exception (i.e. exenatide), the global sales increased each year between 2009 and 2011 (Fig. 3). At >US$4 billion, glatiramer acetate (Copaxone®; Teva Pharmaceutical Industries), a treatment for multiple sclerosis, had the highest global sales. First approved in the USA in 1996, the product's patent is due to expire in 2015 and it might thus be targeted by generics manufacturers. Because Copaxone® is a complex mixture, however, the demonstration of generic equivalence could be challenging. Leuprolide (Lupron®; Abbott Laboratories), first approved in Germany in 1984, achieved global sales of over US$2 billion in 2011.

Five peptide products, octreotide (Sandostatin®; Novartis Pharmaceuticals), goserelin (Zoladex®; AstraZeneca) and liraglutide (Victoza®; Novo Nordisk) had global sales in the US$0.5–1.5 billion range. Octreotide, first approved in 1988, targets somatostatin receptors, and it is a treatment for acromegaly and symptoms in cancer patients. Goserelin, first approved in 1987, targets the gonadotrophin-releasing hormone receptor, and it is marketed for a variety of indications, including management of endometriosis and palliative treatment of advanced prostate and breast cancer.

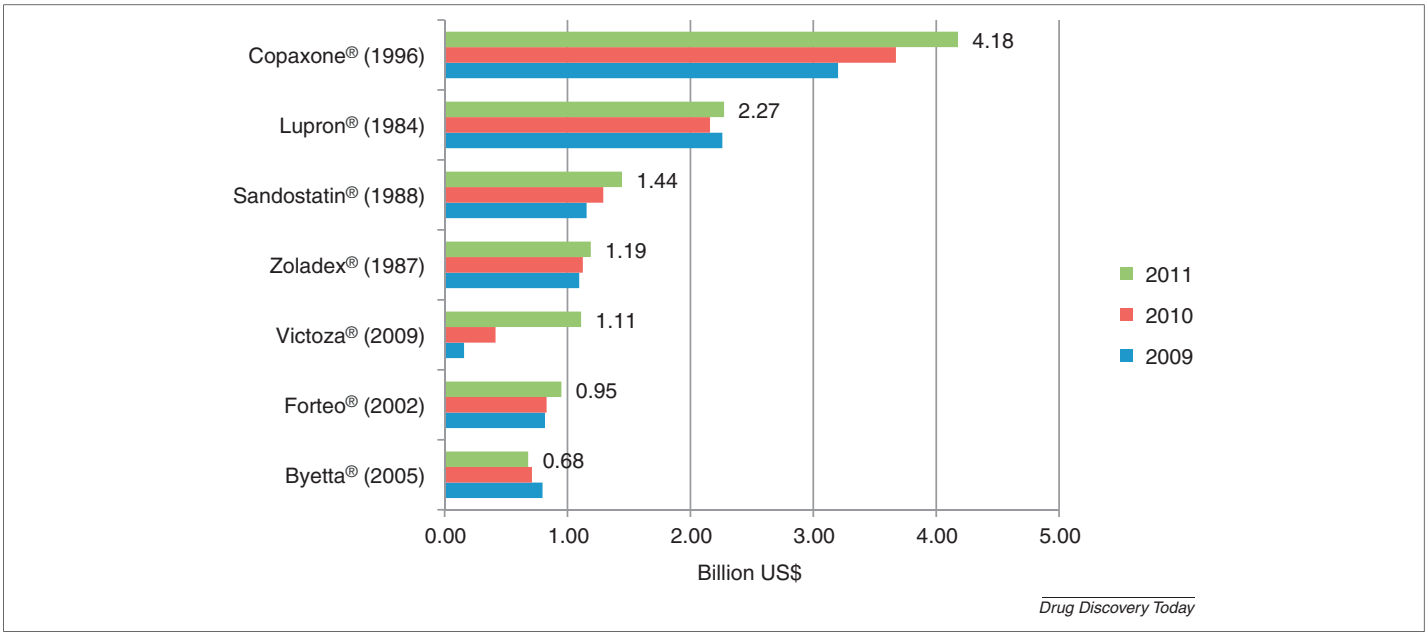

FIGURE 3

Global sales of peptide therapeutics 2009–2011. Year of first marketing approval is indicated after the brand name of each product.

The remarkable increase in global sales for the GLP-1R agonist liraglutide from less than US$500 million in 2010 to over US$1 billion in 2011 might be a harbinger for GLP-1R agonists currently in the pipeline. First approved in 2002, the recombinant parathyroid hormone teriparatide (Forteo®; Eli Lilly) had global sales of nearly US$1 billion in 2011. At US$680 million, the GLP-1R agonist exenatide had the lowest 2011 global sales out of the seven products that we analyzed.

The commercial value of peptide therapeutics that have been marketed for years is well-established, but could substantially increase as recently approved products and those on the horizon gain market share. The type 2 diabetes therapies in the GLP-1R agonist class are emerging as drugs with notable commercial impact that will probably continue to grow along with the type 2 diabetes prevalence. Linaclotide is expected to be a blockbuster with potential worldwide sales of US$1 billion, based on the estimated 63 million people globally affected by irritable bowel syndrome. Teduglutide pricing was recently announced as US$295,000 per patient per year, which could result in annual sales of over US$300 million for this orphan drug. Pasireotide, a unique somatostatin receptor agonist that is the first approved medicine in the USA for Cushing's syndrome and is in Phase III for acromegaly, also demonstrates the growing potential for the development of peptides for rare diseases. Thus, new peptide therapeutics are delivering significant commercial value in a broad range of indications, including those with expanding markets such as diabetes and oncology as well as orphan indications.

Future directions for peptide therapeutics

Although few have entered the clinical pipeline so far, substantial efforts are being directed toward the development of peptides with innovative compositions and mechanisms of action (e.g. PDCs and constrained peptides). Each of these modalities offers opportunities to increase the functionality of peptides and expand the range of targets currently considered suitable for drug development. The concept of targeting cytotoxins or other agents to cells is well-established, and has been successfully applied using antibodies as the targeting modality [41]. As of February 2013, three antibody–drug conjugates (ADCs; i.e. gemtuzumab–ozogamicin, brentuximab–vedotin, trastuzumab–emtansine) had gained approvals from the FDA, although one of these (i.e. gemtuzumab–ozogamicin) was later withdrawn from the market. These marketing approvals resulted from years of research on three crucial components (i.e. the antibody-targeting agent, the linker and the drug). Knowledge gained in the ADC field could enable and accelerate development of PDCs.

Although the term ADC typically refers to molecules in which the drug is specifically a small molecule, the PDC class includes peptides linked to therapeutic agents with a variety of compositions. PDCs in the pipeline include NGR-hTNF (Phase III), AEZS108 (Phase II) and EP100 (Phase II). As we discussed previously, these molecules include a protein (TNF), a small molecule (doxorubicin) and a peptide (CLIP71) as the drug moiety, respectively. The coming years could see a substantial increase in the number and diversity of PDCs in clinical studies. As an example of the possibilities, Shire and Arrowhead Research announced in December 2012 that they have a research collaboration and license agreement to develop PDCs using Arrowhead's homing peptide platform and Shire's therapeutic payloads. Expanding beyond tumor vasculature targeting and increasing the varieties of drug mechanisms of action could extend the utility of PDCs in oncology and other therapeutic areas.

Constraining peptides to specific active conformations can yield high-affinity compounds that are structurally stable and resistant to degradation by proteases, thereby improving their pharmacological properties. The ability to control the 3D shape could also enable design of molecules with more-predictable cell permeability compared with typical unconstrained peptides, opening the potential for the targeting of intracellular processes. Several commercial firms (e.g. Encycle Therapeutics, Lanthio Pharma, Pepscan Therapeutics, PeptiDream, Protagonist Therapeutics, Ra Pharmaceuticals) have developed technologies to produce constrained peptide therapeutics, with recent developments reported from Bicycle Therapeutics and Aileron Therapeutics.

As suggested by their name, the Bicycle Therapeutics platform produces constrained peptides that contain two macrocycles. The molecules are generated from phage display libraries of cysteine-rich peptides [42]. An iterative selection process is used to isolate clones that specifically bind to the target. These clones are then sequenced and the corresponding bicyclic peptide ligands characterized. In proof-of-concept studies, a bicyclic peptide antagonist of urokinase-type plasminogen activator demonstrated notable in vivo stability, and could be conjugated to an albumin-binding peptide [43] or fused to an IgG Fc [44] to yield proteolysis-resistant molecules with extended half-life. In December 2012, Bicycle Therapeutics secured financing for drug discovery in the areas of oncology, metabolic disorders and inflammatory disease.

The basis of the Aileron Therapeutics stapled peptide platform is the use of hydrocarbon staples to enforce α-helices [45–47]. Using this approach, insertion of staples was shown to dramatically improve the PK properties of an HIV-1 fusion inhibitor [48]. As we previously noted, the first stapled peptide entered clinical study in early 2013. Aileron Therapeutics is also evaluating stapled peptides as potential treatments for cancer in preclinical studies. Results for the stapled peptide ATSP7041, a dual inhibitor of MDM2 and MDMX, in xenograft cancer models were presented at the 2012 EORTC-NCI-AACR Symposium on molecular targets and cancer therapeutics [49]. The potential for constrained peptides to target intracellular processes has been noted [47], although advances in this area have been slow to come because of a general lack of understanding of exactly how molecules enter cells. Knowledge gained from study of the CPPs has not been broadly applicable in the design of predictably cell-permeable peptides that have suitable drug-like properties. Better understanding of the basic biology involved in the passive diffusion and active transport of drugs across cell membranes and additional research on the design of constrained peptides might create opportunities to target intracellular processes with these molecules.

Concluding remarks

Although each was the culmination of work done over many years, the marketing approvals of six peptides in 2012 might be seen not

as an end but as a beginning. The unprecedented number of the recent approvals is likely to draw increasing attention to the research and development of peptide therapeutics, which in turn could lead to a substantial increase in the clinical pipeline. At ~130 molecules, the current peptide therapeutics pipeline is robust, but only around one-third that of antibody therapeutics. Our examination of the pipeline revealed notable diversity in the peptides and the indications studied, and highlighted the substantial efforts being made to modify molecular properties to improve functionality. The evolution of the GLP-1R agonists to improve patient compliance and convenience could also be a harbinger for other peptide classes. As evidenced by the recall of peginesatide in February 2013, numerous challenges to the development of peptide therapeutics remain, but the advances discussed here are likely to enrich the peptide pipeline, and could lead to numerous product approvals for peptide therapeutics in the future.

Conflicts of interest

Dr Reichert received financial support from Pfizer and Dr Kaspar was employed by Pfizer during the period the manuscript was prepared. Dr Reichert was a member of the Peptide Therapeutic Foundation's board of directors, who are unpaid, 2008–2012.

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