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Regulatory issues concerning the preclinical testing of synthetic peptides

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Regulatory Issues Concerning the Preclinical Testing of Synthetic Peptides

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Abstract

Any new drug or biological product undergoes rigorous testing in animals and humans for review by the United States Food and Drug Administration (FDA) before it becomes available for human use. A sponsor files an investigational new drug (IND) application with the FDA with supporting animal data, after which testing is continued with humans. There is sufficient guidance from the FDA on new small molecules and biological products as to which preclinical studies are to be conducted. Synthetic peptides present a unique scenario in which a case-by-case approach is needed for the conduct of preclinical studies. For peptides containing components that are already tested for genotoxicity, it is unnecessary to reevaluate them. If such information can be shared with sponsors ahead of the IND application, it can save time and money. There is no research that evaluated the FDA's views on pre-IND consultation. Therefore, through a 10-item questionnaire, FDA reviewers' perceptions on pre-IND consultation, particularly synthetic peptides, were examined. Only four CDER FDA reviewers responded. Three reviewers stated willingness to provide advice to the sponsor through pre-IND discussion and to consult supervisory project managers. Two reviewers agreed that synthetic peptides are to be considered individually for genotoxicity purposes and that pre-testing consultation should be sought. Future research from a larger sample may provide insights on the perceptions of the FDA. Information gleaned from previously approved peptides, however, indicates that there is a wide variability in the type of pre-clinical studies submitted with an NDA (New Drug Application) before progressing to first-in-human studies. However, the routinely submitted studies were single and repeat dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and often studies for impurities.

Keywords: FDA, peptides, IND, preclinical trials

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Chapter 1: Introduction

In the United States, the Food and Drug Administration (FDA) is the institutional body responsible for "protecting the public health by assuring the safety, effectiveness, quality, and security of human and veterinary drugs, vaccines and other biological products, and medical devices" (U. S. Food and Drug Administration, 2018d). Any drug or biological product that is developed for treatment or diagnostic purpose undergoes a rigorous review process by the FDA before it becomes approved for clinical use. A pharmaceutical firm that develops a drug is termed its *sponsor*. A recent report by the Tufts Center for the Study of Drug Development (2014), using the drug development data from 1995–2013 (from 10 firms), indicated that approximately $2.6 billion is spent for each FDA-approved medicine that reaches the US market and that the process from synthesis to approval takes an average of 10 years. Out of 1,442 investigational compounds identified in the research, only 7.1% were approved, while 80.3% had been discontinued and 12.6% were still active by the end of 2013. Of note, the research showed that a significant amount of time (nearly 31 months) and money (nearly 31% of the total expenditure) is spent during the preclinical phase, that is, the phase before the drug is tested in humans (Tufts Center for the Study of Drug Development, 2014).

Peptides are a unique class of drugs that structurally fall between small molecules and proteins and possess unique advantages of high specificity and low toxicity. The first peptide, insulin, was discovered in 1920, and more than 60 peptides have been approved for human use as of 2017 (Lau & Dunn, 2017). Technological advancements in the identification of new receptors and targets involved in disease pathogenesis have paved the way for increased research on peptides for management of disease. However, certain intrinsic properties such as

metabolic instability and the need for parenteral route of administration are major hurdles for peptide development. Chemically synthesized peptides overcome these challenges by the use of non-natural amino acids to improve the metabolic stability, or chemicals such as polyethylene glycols, are used to enhance membrane transportation (Groß, Hashimoto, Sticht, & Eichler, 2015). The most challenging phase for peptide development is the preclinical phase. As peptides do not reach the cellular targets by diffusion like small molecules and exert effects by binding to a cell surface receptor, the determination of their pharmacological and pharmacokinetic parameters through *in vitro* and *in vivo* assays is challenging. (Uhlig, 2014). Although there are guidance documents for sponsors on recommendations for proteins (ICH S6) and small novel chemical entities (NCEs; ICH S1-S5, S7-S8, ICH M3), guidance specific to peptides is limited. Pre-investigational new drug interactions between sponsors and the FDA may help clarify regulatory issues. Given limited literature on the regulation of peptide drug products, this study intended to assess FDA opinions related to the preclinical testing of synthetic peptides.

Chapter 2: Background

Overview of Preclinical Studies Submitted with IND

An investigational new drug (IND) typically consists of non-clinical and clinical components. Key non-clinical components include the preclinical data from the animal pharmacology and toxicology studies, and chemistry, manufacturing, and controls of the investigational drug. Clinical components contain the protocols for the proposed human studies and summaries of previous human experience, if any. Detailed information on the IND application is available in the 21CFR (Code of Federal Regulation) Part 312.

Typically, non-clinical studies are conducted in order to

- evaluate the mode of action of the treatment,
- identify the safe starting dose and dose escalations for the first-in-human studies,
- determine toxic doses and potential target organs,
- assess whether the toxicities are reversible, and, finally,
- determine which safety parameters are to be monitored in human studies (International Conference on Harmonization, 1997).

The nonclinical studies submitted to an IND can be broadly categorized as pharmacokinetics, pharmacology, and animal toxicity studies. *In vivo* testing includes relevant animal models to estimate the lowest dose for therapeutic efficacy (Maralee, 2014). Other non-clinical safety studies include single and repeated dose toxicity studies in two species, reproductive toxicity studies, genotoxicity studies, and studies for special safety concerns such as immunogenicity and carcinogenicity (International Conference on Harmonization, 2009). An overview of the preclinical studies typically conducted as a part of

drug development program is presented in the Table 1. The specific objectives of each type of non-clinical study and a few examples of each type of study are presented in Appendix A.

Table 1

List of Safety Studies

Type of Safety Study	Timing of the Study
Acute Toxicity	Prior to Phase 1, 2 and 3
Sub-acute/Sub-chronic Toxicity	In parallel with Phase I clinical studies
Chronic Toxicity	Concurrently with Phase III clinical trials
Safety Pharmacology	Prior to Phase 1
Genotoxicity	*In vitro*: Prior to Phase 1 *In vivo*: Prior to Phase 2
Developmental and Reproductive Toxicity	Prior to Phase 3 Phase 1 clinical studies (in male volunteers) may start even without the development/reproductive toxicity data, if the treatment does not indicate any testicular damage in safety studies of 2 to 4 weeks' duration
Carcinogenicity	During Phase II and III of clinical development. Usually required for drugs intended for continuous treatment for 6 months or more duration

Note. From "Non-clinical studies in the process of new drug development: Part II: Good laboratory practice, metabolism, pharmacokinetics, safety and dose translation to clinical studies" by E. L. Andrade, A. F. Bento, J.Cavalli, S. K. Oliveira, R. C. Schwanke, J. M. Siqueira, C. S. Freitas, R. Marcon, and J. B. Calixto, 2016, *Brazilian Journal of Medical and Biological Research, 49*, p7–17;

"Chapter 9: Animal use in toxicity studies", *The ethics of research involving animals*. p. 155–167. London: Nuffield Council on Bioethics.

Review Process at FDA

Within the FDA, there are two centers for reviewing an IND application for a therapeutic product: the Center for Drug Evaluation and Research (CDER) and the Center for Biological Evaluation and Research (CBER). A sponsor will submit the IND to CDER or

CBER, depending on the scope of the regulatory supervision of these bodies. Small molecules are covered majorly by the CDER while biological products are primarily reviewed by the CBER (U. S. Food and Drug Administration, 2018b). In 2003, the FDA transferred the regulatory responsibilities for some therapeutic biological products to CDER. These products include monoclonal antibodies and proteins intended for therapeutic use, such as cytokines, enzymes, and other novel proteins, immunomodulators, and targeted biological treatments that alter the production of hematopoietic cells (U. S. Food and Drug Administration, 2018f). CDER/CBER reviews the information with an IND and accepts or rejects the IND (U. S. Food and Drug Administration, 2017d). In 2016, CDER received overall 1669 drug and novel biologic INDs (U. S. Food and Drug Administration, 2017c).

The CDER Offices of New Drugs consists of six Offices and a total of 19 review divisions that undertake the IND reviews. INDs are currently assigned to one review division from the following (U. S. Food and Drug Administration, 2018a):

- Office of Drug Evaluation I – Division of Cardiovascular and Renal Products; Division of Neurology Products; Division of Psychiatric Products.
- Office of Drug Evaluation II – Division of Metabolic and Endocrineo Products; Division of Pulmonary, Allergy, and Rheumatology Products; Division of Anesthesia, Analgesia, and Addiction Products.
- Office of Drug Evaluation III – Division of Gastroenterology and Inborn Errors Products; Division of Bone, Reproductive, and Urologic Products; Division of Dermatology and Dental Products.

- Office of Drug Evaluation IV – Division of Nonprescription Clinical Evaluation; Division of Medical Imaging Products; Division of Pediatric and Maternal Health Products.
- Office of Antimicrobial Products (OAP) – Division of Anti-Infective Products; Division of Antiviral Products; Division of Transplant and Ophthalmology Products.
- Office of Hematology and Oncology Products (OHOP)– Division of Oncology Products (one and two); Division of Hematology Products; Division of Hematology Oncology Toxicology.

FDA Review Process in Brief

When an IND application is received, the division director appoints the cross-discipline team leader based on the content of application; most often the choice is a medical officer, who is also responsible for the review of clinical section (U. S. Food and Drug Administration, n.d.). The other review team members are

- project managers, who are the primary contacts with the sponsor and who prepare the review plan, coordinate the review team activities, monitor the review status, maintain up-to-date information on milestones, and assure a timely review;
- a medical officer, who reviews clinical studies;
- a pharmacology/toxicology specialist, who reviews all nonclinical (animal) studies;
- statisticians, who review protocols and the statistical analysis plan;
- clinical pharmacology/biopharmaceutics reviewers, who evaluate pharmacokinetic and pharmacodynamics of the study drug; and
- chemists, who evaluate drug chemistry, manufacturing and controls, stability profile, and other chemistry issues (U. S. Food and Drug Administration, 2015).

FDA Guidance for Sponsors

The FDA recommends that sponsors follow the requirements in guidance documents published by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH). These documents can help formulate essential studies to be submitted with an IND to gain approval for first-in-human trials. The requirements for the conduct of each type on non-clinical studies are listed in specific ICH guidance documents, listed in Table 2, which also extends further (U. S. Food and Drugs Administration, 2018e; International Conference on Harmonization, 2009)

Table 2

List of ICH Guidance Documents by Type of Non-clinical Study

***Non-clinical Tests* – Related ICH guidance**	**ICH guidance document**
Pharmacokinetic Studies:	
Guidance on toxicokinetics: Assessment of systemic exposure in toxicity studies	ICH S3A
Guidance on pharmacokinetics: repeated dose tissue distribution studies	ICH S3B
Chronic Toxicity Studies:	
Guidance on duration of chronic toxicity testing in animals (rodents and non-rodent toxicity testing)	ICH S4
Genotoxicity Studies:	
Genotoxicity testing and data interpretation for pharmaceuticals intended for human use	ICH S2 (R1)
Guidance on assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk	ICH M7

Table 2 *continued*

Carcinogenicity Studies: Guidance on • Need for carcinogenicity and • Testing for carcinogenicity of Pharmaceuticals • Dose selection for carcinogenicity studies of pharmaceuticals	ICH S1A, S1B, S1C (R2)
Safety Pharmacology Studies Guidance on • Safety pharmacology studies for human pharmaceuticals • Nonclinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals	ICH S7A, S7B
Reproductive Toxicity Studies Guideline on Detection of Toxicity to Reproduction for Human Pharmaceuticals	ICH S5
Immunotoxicity Studies Guidance on immunotoxicity studies for human pharmaceuticals	ICH S8

Note. All guidance documents were developed within the Safety Implementation Working Group of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

There are multiple opportunities for the drug sponsor to meet with the FDA team in the drug developmental process. Such interactions can help sponsors not only to reduce chances of IND rejection but also to learn of current issues. One such opportunity is the pre-IND meetings. These can reduce time to market in many ways, such as by identifying and avoiding unnecessary studies, confirming designs of the needed studies, minimizing costs, and allowing early interactions/negotiations with the FDA. The pre-IND meeting request

must be submitted at least four weeks earlier than the scheduled date and along with a pre-IND package containing all necessary background information and the list of questions that the sponsor requires the FDA to answer. Pre-IND meetings are useful for sponsors for studies with scientific or regulatory issues related to clinical trial design, toxicity, unique metabolites, non-standard or novel formulations, dosing limitations, species suitability, and immunogenicity (National Heart, Lung, and Blood Institute, 2013). There is a specific FDA guidance document that talks about the conduct of formal meetings (U. S. Food and Drug Administration, 2017b).

Prior to a pre-IND meeting, the sponsor may also have an informal meeting with the FDA team, through a pre-pre-IND teleconference. This will not reduce the necessity of a pre-IND meeting but can help the sponsor get possible solutions related to preclinical issues. For example, the selection of animal model for the preclinical study can be discussed (Feigal et al., 2012).

Peptides and Synthetic Peptides

Peptides have been a unique, rapidly growing class of treatments since the advent of insulin in the early 1920s (Banting, Best, Collip, Campbell, & Fletcher, 1922). Across the United States, Europe, and Japan, more than 60 peptide drugs were approved before 2017, and more than 150 are under active clinical development (Lau & Dunn, 2017). Peptides have been recognized as promising therapeutic agents for the treatment of various conditions such as cancer, and, metabolic, infectious, or cardiovascular diseases (Vlieghe, Lisowski, Martinez, & Khrestchatisky, 2010; Lau & Dunn, 2017). Special advantages that peptides show over other drugs include being highly versatile, target-specific, less toxic, and able to act on a wide variety of targets (Fosgerau & Hoffmann, 2015; Vlieghe et al., 2010), which are directly

responsible for greater success rate than small molecules (approval rate of around 20% versus 10%; Lax, 2013; Uhlig et al., 2014). The list of FDA approved peptides is presented in Appendix A (Usmani et al., 2017).

Initially, life-saving peptides such as insulin and ACTH were isolated from natural sources. The feasibility of chemical synthesis of peptides in 1950s enabled the introduction of synthetic oxytocin and vasopressin. Further, the recent technological developments in the genomic era helped in identification and characterization of peptide hormone receptors, and novel ligands for these receptors are being actively explored (Feigal et al., 2012).

The intrinsic limitations for peptides include metabolic instability, that is, the inability to withstand 600 proteases in the human body (Lopez-Otin & Matrisian, 2007) and restriction to the parenteral route of administration. Chemically synthesized peptides can overcome both of these challenges by incorporating additional entities, such as non-natural amino acids, to improve the metabolic stability, or other chemical entities, such as polyethylene glycols, to enhance membrane transportation (Groß et al., 2015).

During the drug developmental process, the most challenging phase for peptides is the preclinical phase. Conventional smaller therapeutic molecules reach their cellular targets by diffusion, but peptides enter cells through the surface receptors, and therefore, determining their *in vitro* and *in vivo* pharmacology from immunological assays becomes challenging (Uhlig et al., 2014). For example, 4F peptides have demonstrated potent anti-inflammatory, anti-oxidant, and atheroprotective effects in preclinical models of apoE null mice and in human aortic cell cultures but failed to show the same effects in human trials. This could be attributed to the differences in the composition of lipid-associated proteins between humans and mice (Recio, Maione, Iqbal, Mascolo, & De Feo, 2016). Peptide-based

therapeutics have a higher chance of clearing the early clinical trials than other drugs, once they overcome the IND hurdles (Otvos, 2014).

Importance of FDA guidance on preclinical studies of synthetic peptides. Peptides fall under a distinct category of treatments, and there is no FDA guidance specific to the preclinical testing of peptides. The current biopharmaceutical guidance documents are used to determine the recommended battery of essential preclinical tests. The ICH S6 consists of recommendations for biological therapeutics while other documents, such as ICH S1-S5, S7-S8, ICH M3, are for smaller molecule drugs. The areas of uncertainty for peptide drug development, for which a pre-IND consultation would greatly help, are described below.

Species selection. Typically, it is recommended that toxicology testing be performed in two different species; the most commonly used are rats and dogs (Kingham, Klasa, & Carver, 2010). As biologics show tissue-specific activity, conducting such studies in a pharmacologically irrelevant species will be inappropriate. In those cases, sponsors may utilize homologous proteins or transgenic animals that express human receptor or other animal models (Kingham et al., 2010).

Immunogenicity is another factor for species selection, particularly for vaccine peptides. Sponsors should always note that animal data are not truly indicative of human immune response. Elevated antibody production during repeat-dose toxicity studies may not necessarily be the rationale for the termination of the preclinical studies unless observed in a large proportion of animals (Kingham et al., 2010).

Genotoxicity. Previously, in a typical small molecule preclinical testing approach, genotoxicity testing was often conducted. However, recent regulatory guidance documents have been amended and now state that genotoxicity testing is not necessary with biological

products. This is because they are not directly associated with any known genetic toxicity risk (International Conference on Harmonization, 2011). But in instances where the biologic product has any molecule with such risk, genetic toxicity testing might be needed (Vugmeyster, Xu, Theil, Khawli, & Leach, 2012).

Synthetic peptides present a special scenario for genotoxicity testing. If the test peptide contains exclusively natural amino acids, testing may not be necessary. Also, testing may not be necessary for peptides that contain already tested non-natural amino acids, linkers, and non-linker components. However, if such peptides have been modified for better cellular absorption, the *in vivo* genotoxicity testing may be necessary. Linkers that can be potentially mutagenic impurities need to be evaluated as per ICH M7 (Thybaud et al., 2016). Testing for such process-related impurities is the key difference between the biotechnologically-derived and chemically-synthesized peptides (Heidel & Page, 2010).

Pre-IND and "pre-pre-IND" meetings are the best opportunities for the sponsors to discuss any preclinical issues with the FDA before IND filing. These meetings can help sponsors provide adequate information with the IND and avoid clinical holds from the FDA (Feigal et al., 2012).

Purpose of the Study

Although many published sources recommended that pre-IND consultation would be beneficial for sponsors to aid the therapeutic biologic development process, there is no research that captured the FDA's perspective on this issue. Synthetic peptides have been a key research area in recent years with several preclinical issues, as highlighted in previous sections. Any direct evidence captured from the FDA reviewers' viewpoint could further help sponsors plan better for the consultation program. Hence, this study was conducted to assess

FDA opinions related to synthetic peptides in two ways. First, a survey was conducted to understand the FDA reviewers' responses to the questions related to the conduct of preclinical studies, with some specifically on synthetic peptides. Second, FDA documentation was reviewed to summarize the non-clinical studies from the recently approved synthetic peptides.

Research Questions

The first part of the study aimed to address the following through this survey:

- What are the preclinical toxicology requirements for synthetic peptides?
- How can researchers obtain input?
- What suggestions might FDA reviewers have to resolve this question?

The second part of the study aimed to address the following:

- What non-clinical studies have been submitted with INDs for recently approved synthetic peptides?

Chapter 3: Methods

Part 1 – Online Survey of FDA Reviewers

The study used an e-mail survey with a 10-item multiple-choice questionnaire. As this research focused on understanding the perceptions of the FDA on regulatory issues of synthetic peptides, FDA reviewers were considered the targeted study population. In the questionnaire, the first three items captured the background profile and experience of the FDA employee. The next seven items included specific questions related to the synthetic peptides. Appendix B contains the questionnaire. Space was left at the end of the questionnaire for free text suggestions or feedback from survey participants.

The survey questions were uploaded into the Google survey tool, Google forms, and the link was saved by the researcher. After receiving the approval of the University Human Subjects Review Committee at Eastern Michigan University (Appendix C), the Google form link with the survey questions was forwarded to one of the FDA employees. This employee later directed it individually to other FDA employees, from his contacts in the FDA, via their official e-mail IDs. Overall, the questionnaire was forwarded to approximately eighty FDA reviewing or supervisory pharmacologists.

Part 2 – Review of Previously Submitted Non-Clinical Studies

In the second part of the study, data from two recent review articles by Vlieghe and colleagues (2010), and Lau and Dunn (2017) that contained the names of approved synthetic peptides across the world were reviewed. In the review article by Vlieghe et al. (2010) the authors tabulated the information on peptides approved in the US, Europe, and Japan, with specific details on peptide length (number of amino acids) and sequence, and the specific indications. In the review article by Lau and Dunn (2017), the authors also summarized the

status in the US, Europe, and Japan on approved peptides, as well as their route of administration and targeting receptors in the supplementary table of the published paper. Each peptide identified from the two articles was then checked for US approval dates, number of amino acids, manufacturing process, and whether synthetic or not, from the FDA website (U. S. Food and Drug Administration, 2018c) and the Drug Bank online database (DrugBank, 2018). For the current research, any polymer with 40 or fewer amino acids was considered a peptide, as adopted by the FDA. Of them, only synthetic peptides with 40 or fewer amino acids approved by FDA composed the peptide set of interest for this study.

For synthetic peptide drugs approved between 2007 and 2017, the non-clinical pharmacology review information was obtained from the FDA website (U. S. Food and Drug Administration, 2018c). From each PDF document, information with respect to non-clinical studies was extracted and summarized.

Chapter 4: Results

Part 1 – Online Survey of FDA Reviewers

Of the 80 recipients of the questionnaire email, only four FDA reviewers responded. The survey responses are presented in Table 3. Out of four, three respondents were reviewing pharmacologists and one was a supervisory reviewer. All of them were reviewers at the CDER. Experience at the FDA was more than 10 years for three respondents, while a single respondent's experience was between 6 and 10 years.

Most reviewers were willing to provide advice to sponsors prior to submission but only with a pre-IND meeting request, whereas one reviewer was against providing any informal advice. Two preferred face-to-face conversation as the best way of communication with sponsors regarding any advice. Three of the four respondents recommended contacting the supervisory project manager for advice. Two respondents agreed that pre-testing consultation was necessary for synthetic peptides and that each compound should be treated separately. Also, two reviewers suggested that more than two months' time should be allowed for receiving input from the FDA before beginning toxicology tests of synthetic peptides. All of the reviewers agreed that pharmacological differences between human and animal models are a concern for selection of species for toxicological studies. In that case, two reviewers recommended pre-testing consultation with FDA, while two stated that animals should exhibit the same pharmacology as humans for toxicity testing. Finally, two reviewers claimed to have had personal experiences in dealing with issues pre-IND, but not as the specific ones mentioned in the survey. One reviewer had an experience similar to those in the questionnaire.

Table 3

Responses to the Survey

Question	n	%
1. Position at FDA		
Reviewing Pharmacologist	3	75%
Supervisory Pharmacologist	1	25%
2. Review Center		
CDER	4	100%
3. Years at FDA		
6 to 10	1	25%
Greater than 10	3	75%
4. Are you willing/able to provide informal advice to sponsors prior to submission of an IND?		
Yes, but a pre-IND meeting request and supporting documentation must be submitted	3	75%
No, an active IND must be on file	1	25%
5. The best way to obtain advice prior to IND submission is via		
Teleconference to discuss previously submitted materials	1	25%
Face-to-face meeting to discuss previously submitted materials	2	50%
Email replies from a reviewer to very specific questions	1	25%
6. The appropriate contact to request pre-IND advice is		
The supervisory project manager	3	75%
Any divisional project manager	1	25%
7. Synthetic peptides should be considered, for genotoxicity purposes		
To be handled like a biologic product	1	25%
To vary by compound. Pre-testing consultation should be sought	2	50%
Unknown	1	25%
8. If human synthetic peptides or biologics do not show the same pharmacology in animal models as they do in human tissue cultures, is this a concern for selection of species for toxicology testing?		
Yes, the animal model used for toxicity testing must exhibit the same pharmacology as that expected in humans	2	50%
Pre-testing consultation with the Review Division should be sought	2	50%

Table 3 *continued*

Question	n	%
9. If pre-testing consultation is required before beginning toxicology test, how much time should be the sponsor build into their planning to allow appropriate input from agency?		
1 month	1	25%
2 months	1	25%
More than 2 months	2	50%
10. Have you had personal experience dealing with issues like those mentioned in this survey?		
Yes for issues pre-IND, but not those specific ones mentioned here.	2	50%
Yes	1	25%
No	1	25%

Part 2 – Review of Previously Submitted Non-Clinical Studies

Out of more than 60 approved peptide drugs identified from the two papers, the final set included 41 peptides that were chemically synthesized, approved by the FDA, and had 40 or fewer amino acids. These peptides are listed in Table 4.

Table 4

FDA-Approved Synthetic Peptides

S. No	Synthetic peptide drug	FDA approval year	Number of amino acids	Indication	Status
1	Corticotropin	1950	39	Diagnosis - adrenocortical insufficiency	Discontinued
2	Lypressin	1961	9	Central diabetes insipidus, Cushing's syndrome	Discontinued
3	Tetracosactide	1970	24	Diagnosis - adrenocortical insufficiency	Prescription
4	Desmopressin	1978	9	Central diabetes insipidus, nocturnal enuresis, nocturia, and stoppage of bleeding or hemorrhage in haemophilia A patients	Prescription
5	Oxytocin	1980	9	Initiation or improvement of uterine contractions, and control postpartum hemorrhage	Prescription
6	Saralasin acetate	1981	8	Hypertension	Discontinued
7	Gonadorelin	1982	10	For evaluating gonadotropes of the anterior pituitary and residual gonadotropic function of the pituitary following therapy	Discontinued
8	Enalapril	1985	3	Hypertension	Prescription
9	Calcitonin salmon	1986	32	Postmenopausal osteoporosis, Paget's disease, hypercalcaemia	Prescription
10	Calcitonin (human)	1986	32	Postmenopausal osteoporosis, Paget's disease, hypercalcaemia	Discontinued

Table 4 *continued*

S. No	Synthetic peptide drug	FDA approval year	Number of amino acids	Indication	Status
11	Lisinopril	1987	3	Acute myocardial infarction, heart failure, hypertension	Prescription
12	Octreotide Acetate	1988	8	Acromegaly, neuroendocrine tumors	Prescription
13	Goserelin	1989	10	Advanced prostate cancer, breast cancer	Prescription
14	Nafarelin	1990	10	Central precocious puberty, endometriosis, uterine fibroids, ovarian stimulation in *in vitro* fecundation	Prescription
15	Histrelin	1991	9	Advanced prostate cancer, central precocious puberty	Prescription
16	Glatiramer	1996	Random mixture	Relapsing-remitting multiple sclerosis	Prescription
17	Eptifibatide	1998	7	Acute coronary syndrome, unstable angina undergoing PCI	Prescription
18	Ganirelix	1999	10	Inhibition of premature leutinizing hormone surges in controlled ovarian hyperstimulation	Prescription
19	Triptorelin	2000	10	Advanced prostate cancer, central precocious puberty, endometriosis, uterine fibroids, ovarian stimulation in *in vitro* fecundation	Prescription
20	Cetrorelix	2000	10	Inhibition of premature LH surges in women Undergoing controlled ovarian stimulation	Prescription
21	Bivalirudin	2000	20	Anticoagulant for unstable angina patients undergoing angioplasty	Prescription
22	Leuprolide acetate	2002	9	Advanced prostate cancer, breast cancer, central precocious puberty	Prescription
23	Abarelix	2003	10	Advanced prostate cancer	Discontinued
24	Enfuvirtide	2003	36	AIDS	Prescription
25	Ziconotide	2004	25	Severe chronic pain	Prescription
26	Pramlintide	2005	37	Type 1 and 2 diabetes	Prescription
27	Exenatide	2005	39	Type 2 diabetes mellitus	Prescription
28	Lanreotide	2007	8	Acromegaly, carcinoid syndrome	Prescription
29	Degarelix	2008	10	Advanced prostate cancer	Prescription
30	Liraglutide	2010	31	Type 2 diabetes	Prescription
31	Icatibant	2011	10	Hereditary angioedema	Prescription
32	Lucinactant	2012	21	Neonatal respiratory distress syndrome	Discontinued
33	Linaclotide	2012	14	Constipation-dominant irritable bowel syndrome, chronic idiopathic constipation	Prescription
34	Pasireotide	2012	6	Cushing's disease, specifically for patients not eligible for pituitary surgery	Prescription
35	Carfilzomib	2012	4	Progressive multiple myeloma after treatment with bortezomib and an immunomodulatory agent	Prescription
36	Etelcalcetide	2017	7	Secondary hyperparathyroidism in chronic kidney disease patients on hemodialysis	Prescription
37	Plecanatide	2017	16	Chronic idiopathic constipation	Prescription
38	Abaloparatide	2017	34	Postmenopausal osteoporosis	Prescription
39	Semaglutide	2017	31	Type 2 diabetes mellitus	Prescription
40	Macimorelin	2017	2	Diagnosis - adult growth hormone deficiency	Prescription
41	Angiotensin II	2017	8	Increase blood pressure in patients with sepsis or other critical conditions	Prescription

Note. From "Synthetic therapeutic peptides: science and market" by P. Vlieghe, V. Lisowski, J. Martinez, and M. Khrestchatisky, 2010, *Drug Discovery Today, 15*, p. 40–56;

"Therapeutic peptides: historical perspectives, current development trends, and future directions" by J. L. Lau and M. K. Dunn, 2017, *Bioorganic & Medicinal Chemistry, 26*, p. 2700–2707;

"Therapeutic peptides" by F. Albercio and H. Kruger, 2012, *Future Medicinal Chemistry, 4, p. 1527–31;*

"2017 FDA peptide harvest" by O. Al Musaimi, D. Al Shaer, D. L. Torre, and F. Albericio, 2018, *Pharmaceuticals (Basel), 11*, p. 7.

Among the listed peptides, the 14 that were approved between 2007 and 2017 (lanreotide, degarelix, liraglutide, icatibant, lucinactant, linaclotide, pasireotide, carfilzomib, etelcalcetide, plecanatide, abaloparatide, semaglutide, macimorelin, and Angiotensin II) were further researched by an examination of the non-clinical FDA reviews. There was a non-uniform distribution of the FDA approvals over the time period of 2007–2017: three were approved between 2007–2010, five approved between 2011–2012, and six approved in 2017. No synthetic peptides were approved in the years 2013–2016. The ideal way to present such heterogenous non-clinical study data is through tables and a maximum of only four could be accommodated in one table. Hence, these safety studies submitted with the NDA (New Drug Application) for these peptides summarized in Tables 5–8 in a chronological order: Table 5 for peptides approved in 2007–2010, Table 6 for those approved in 2011 and 2012 (excluding lucinactant), and Tables 7 and 8 for those approved in 2017 (three peptides as Part A and three peptides as Part B). For the majority of these peptides, the following categories of general toxicology studies were conducted: single-dose toxicity, repeat-dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and special toxicology studies.

The series of non-clinical tests for Lucinactant were not included in Tables 5–8, since the non-clinical tests were very different from all other synthetic peptide drugs considered. Lucinactant was an intratracheal drug with targeted place of action (lungs) to treat acute respiratory distress in neonates. The non-clinical program of lucinactant included the following toxicology studies: single dose in newborn rabbits, 7 days in adult rats, 4 days in rabbits, 14 days in newborn rabbits, a single dose in premature monkeys, immunogenicity in

monkeys, and systemic anaphylaxis in guinea pigs, preterm lambs, and fetal rabbits assay. The drug has been discontinued, however, to support the development of another aerosolized KL4 surfactant for the same indication by the sponsor (Windtree Therapeutics, 2015).

Table 5

Safety Studies of Synthetic Peptides Approved Between 2007 and 2010

	Lanreotide	**Degarelix**	**Liraglutide**
Single Dose	Mice, rats (previous*)	Mice, rats, monkeys	Mice, rats
Repeat Dose			
1 week			Rats
2 weeks		Rats, monkeys	Monkey
4 weeks		Rats, monkeys	Mice, rats, monkeys
13 weeks		Rats, mice	Mice, rats, monkeys
26 weeks	Rats, dog (previous*) Rats, dog (new formulation)	Rats	Rats
52 weeks			Monkeys
24 months	Dog (previous*)		
Genetic Toxicology tests			
Ames test	S. typhi and E.coli (previous*)	S. typhi and E.coli	S. typhi and E.coli
In vitro DNA damage in mammalian cells	Cultured human peripheral lymphocytes (previous*), L5178Y/TK+/- mouse lymphoma cells	L5178Y/TK+/- mouse lymphoma cells	Cultured human peripheral lymphocytes
In vivo test for genetic damage	Mouse micronucleus tests (previous*)	Rat micronucleus tests	Rat micronucleus tests
Other genotoxicity tests	Mutations in tissues of Muta™ Mice		
Carcinogenicity	104 weeks rats, mice	104 weeks rats, mice	104 weeks rats, mice
Reproductive toxicology			
Segment 1 – Fertility	Rats (previous*), Rats (present)	Rats	
Dose range finding	Rats, rabbits (previous*)		Rabbits

Table 5 *continued*

	Lanreotide	Degarelix	Liraglutide
Segmental (1/2) - reproductive		Rats	Rats
Segment 2 - teratology	Rats, rabbits (previous*)		Rabbits
Pre- and Post-natal Development		Rats, rabbits	Rats
Other Tests		None	
Local toxicity	Rabbits, monkeys, minipigs		Pigs
Mechanistic studies			For t-cell tumors
Impurities			4 weeks rat toxicity study
Other studies			Toxicology studies with exenatide for comparison

Note. IND, investigational new drug; NDA, new drug application.

*"Previous" refers to studies submitted with previous IND submission or NDA submissions for the same molecule.

Table 6

Safety Studies of Synthetic Peptides Approved During 2011–2012

	Icatibant	**Linaclotide**	**Pasireotide**	**Carfilzomib**
Single Dose	Mice, rats, dog (previous*)	Rats, monkeys	Mice, rats	Rats, Monkeys
Repeat Dose				
5 days			Rats, dog	Monkeys
1 week			Rats	
2 weeks	Rats	Rats, monkeys	Mice, rats, dog, monkeys	
3 weeks				Rats
4 weeks	Dogs	Mice (Common study for drug and impurities)	Monkey, mice, rats	Monkeys
13 weeks	Rats, dogs	Mice, rats, monkeys		Rats
26 weeks		Mice	Rats	Rats
39 weeks		Monkeys	Monkeys	Monkeys
Dose Ranging Study	Dogs		Rats, monkey (2 week)	
Genetic Toxicology tests				
Ames test	S. typhi	S. typhi, E coli	S. typhi	S. Typhi and E. coli
In vitro DNA damage in mammalian cells	Cultured human lymphocytes	Cultured human lymphocytes	Cultured human lymphocytes	Cultured human lymphocytes
In vivo test for genetic damage	Rat micronucleus test	None	Rat micronucleus test	Mouse micronucleus test
Other genotoxicity tests		None		
Carcinogenicity	None	104 weeks rats, mice	104 weeks rats, 6 months transgenic mice	No carcinogenicity studies as per ICH S9
Reproductive toxicology				
Segment 1 – Fertility		Rats	Rats	

Table 6 *continued*

	Icatibant	Linaclotide	Pasireotide	Carfilzomib
Dose range finding (Segment 2)			Rats, rabbits	
Segment 2 - teratology	Rats, rabbit (previous*)	Mice, rats, rabbits	Rats, rabbits	Rats, rabbits
Segment 3 - Pre- and Post-natal Development	Rats	Rats	Rats	
Fertility and early embryonic	Combined with 13-week toxicity test		*In vitro* assessment using rat whole embryo culture	
Other Tests				
Local toxicity	Rabbit		Ocular rabbit, dermal rabbit, Rabbit/rat IM tolerance	
	In vitro hemolysis test		Rat immunotoxicity	
	Hyperimmunization studies in the rat, dog and monkey			
Impurities	For two degradants, in mice, single dose study Safety qualification of impurities – IV toxicity test, Ames test, Chromosome aberration test	4-week toxicity for impurities, Ames test for impurities (also mentioned above)	4-week toxicity test for impurities, Ames test for impurities, Chromosome aberration test for impurities	
Others		Tested in juvenile mice and juvenile rabbits	Phototoxicity	Three-part study to investigate effect of proteasome inhibition on endotoxin challenge in mice

Note. IND, investigational new drug; NDA, new drug application.

* "Previous" refers to studies submitted with previous IND submission or NDA submissions for the same molecule.

Table 7

Safety Studies of Synthetic Peptides Approved in 2017 – Part 1

	Plecanatide	**Abaloparatide**	**Etecalcitide**
Single Dose	Mice, monkeys, rats	Mice, rats	Not conducted
Repeat Dose			
5 days	Mice, Monkeys (3 days)	Monkeys (3 days)	
1 week	Mice, rats (7/14 days)		Rats, Dogs
2 weeks	Rats (7/14 days), monkey		
4 weeks	Mice, monkeys	Rats, monkeys	Rats, Dogs
13 weeks	Mice, rats, monkeys	Rats, monkeys	Rats, Dogs
26 weeks	Mice	Rats	Rats, Dogs
39 weeks	Monkeys	Monkeys	Dogs
Genetic Toxicology tests			
Ames test	E.coli, S.typhi	E.coli, S. typhi,	S.typhi and E.coli strains
In vitro DNA damage in mammalian cells	L5178Y/TK+/- mouse lymphoma cells	Cultured human peripheral lymphocytes	Cultured human peripheral lymphocytes, CHO-K1 cells, Chinese hamster lung fibroblast (V79) cells
In vivo test for genetic damage	Mouse micronucleus test	Mouse micronucleus test	Rat micronucleus test
Other genotoxicity tests			4 week muta™ mouse test
Carcinogenicity	104 weeks rats, mice	104 weeks rats	104 weeks rats, 6 months transgenic mice
Reproductive toxicology			
Segment 1 – Fertility	Mice	Rats	Rats (combined Segment 1, 2)

Table 7 *continued*

	Plecanatide	Abaloparatide	Etecalcitide
Dose range finding (Segment 2)	Mice, rabbits		Rats, rabbits
Segment 2 - teratology	Mice, rabbits		Rats, rabbits
Pre- and Post-natal Development	Mice		Rats
Other Tests		Phototoxicity	
Local toxicity		Local tolerance – rabbit, rat, monkey	Dogs
	Juvenile toxicity – mice, single dose, 7 days, 14 days, 14 or 13 weeks		Hemolysis testing blood
Impurities		Rats (2 weeks, 4 weeks) with unknown degradant, standard battery of genotoxicity for impurities	4 weeks rats for mixture and impurities, 4 weeks dogs comparing 2 lots, Ames test for sodium isopropyl sulphate
Mechanistic Studies			1.etelcalcetide and structurally related peptides were evaluated for mutagenic activity, 2. For metabolite formation, 3. L-cysteine for mutagenic potential, and 3 more studies on mutagenicity
Antigenicity	Rabbits (105 days, 136 days)		

Note. IND, investigational new drug; NDA, new drug application.

Table 8

Safety Studies of Synthetic Peptides Approved in 2017 – Part 2

	Semaglutide	**Macimorelin**	**Angiotensin-II**
Single Dose	Mice, rats (previous*)	Rats, dogs	Rats (Lit Search)
Repeat Dose			Rats, rabbits (Lit Search)
5 days		Rats, dogs	
2 weeks	Mice (previous*), Monkey (previous*)	Rats	
4 weeks	Rats	Rats, dogs	
13 weeks	Rats, Mice, rats (previous*), monkey (previous*)		
26 weeks	Rats		
52 weeks	Monkeys		
Dose Ranging Study	8-18 days mice (previous*) 14-25 days (previous*)		
Genetic Toxicology tests			Lit Search
Ames test	E. coli, S. typhi (previous*, present)	S. typhi (previous*)	
In vitro DNA damage in mammalian cells	Cultured lymphocytes (previous*, present)	L5178Y T/K+/- mouse lymphoma cells, CHO-K1 cells	
In vivo test for genetic damage	Rat micronucleus test	Not conducted	
Other genotoxicity tests		DEREK evaluation and MCASE evaluation for mutagenicity and genotoxicity of macimorelin and impurities	

Table 8 *continued*

	Semaglutide	Macimorelin	Angiotensin-II
Carcinogenicity	Rats, mice – 2 year *In vitro* GLP-1R activation in rat thyroid C cell-line Mice (single dose), Rats (6 weeks dose) for calcitonin levels	Not done as it is single dose product and the drug pharmacology/toxicology is known	Lit search
Reproductive toxicology		Not done as it is single dose product and the drug pharmacology/toxicology is known	Lit Search
Segment 1 – Fertility	Rats		
Segment 2 - teratology	Rats, rabbit, monkey, rats (juvenile toxicity) rabbit (previous*) monkey (previous*), juvenile toxicity (previous*, animal not mentioned)		
Pre- and Post-natal Development	Monkey		
Other Tests			
Local toxicity	Pigs (previous*), rabbits (previous*)		
Mechanistic Studies	For embryotoxicity to explore species specificity Conducted whole embryo cultures and studies in yolk sacs of untreated rats, monkeys		

Note. IND, investigational new drug; NDA, new drug application.

*"Previous" refers to studies submitted with previous IND submission or NDA submissions for the same molecule.

Chapter 5: Discussion

Part 1 – Online Survey of FDA Reviewers

All of the survey respondents were well experienced, with 6 or more years of association with the FDA; hence, their thoughts on the survey questions are likely representative of FDA pharmacologists. Three of four reviewers agreed to provide informal advice to sponsors through a pre-IND meeting and supporting documentation, highlighting the significance of such meetings. However, there are no published data that present the number/proportion of sponsors approaching FDA for a pre-IND meeting advice. Of the four, only one reviewer agreed that teleconferencing was the best way to obtain advice prior to IND submission. This may imply that pre-pre-IND meetings, which are usually conducted through teleconferences, may not be of great value to the sponsors. There are some specific advantages for a telephonic conference over the face-to-face meeting: Scheduling is easier, and it is cheaper and more accessible to staff (Yetter, 2005). Two of the four reviewers recommended that meeting face-to-face is the best way. Face-to-face meetings are preferred only if such type of interaction is warranted, such as in case of issues concerning scientific, clinical, and regulatory aspects of the new products (Novak, Ruckman, & Trent, 2009). The sponsor can request a meeting and the FDA can take a call to provide a written response or accept a face-to-face meeting based on the questions posed to the FDA (Vaknalli, 2017). A majority (3/4) of the respondents agreed that the appropriate contact to request pre-IND advice was the supervisory project manager. The FDA guidance document that consists of best practices for communications between sponsors and FDA mentioned that the regulatory project manager is the ideal primary point of contact (U. S. Food and Drug Administration, 2017a).

In the questions specific to synthetic peptides, two of the four CDER reviewers suggested that the synthetic peptide evaluation needs to vary by compound and that pre-testing consultation should be sought. This is inconsistent with the article by Thybaud et al. (2016), in which the genotoxicity was described as being assessed only in the presence of non-natural amino acids or linkers or other components that were not previously assessed for genotoxicity. As such information gets updated frequently, meeting the FDA before toxicity testing can greatly help eliminate unnecessary studies and confirm the study design. Related to the question on the use of animal models for toxicology testing of synthetic peptides, two reviewers agreed that pre-testing consultation should be sought when human tissue cultures and animal models do not exhibit same pharmacology. Two reviewers answered by saying that animal models should show the same pharmacology. In cases when there are pharmacologically irrelevant species, there are alternatives: performing tests in only the relevant species, use of transgenic animals, and so on. These can be discussed in a pre-IND consultation to determine which tests can be performed. To the question on a timeline for getting input from the agency, three reviewers stated that a period of at least 2 months before beginning the toxicology tests was reasonable for appropriate input from the FDA. As per the FDA meeting guidance, pre-IND meetings need to be scheduled within 60 days of the day of receipt of the sponsor's meeting request. However, the time limit for sponsors to share the materials is 30 days before the meeting, which leaves the agency with only 30 days to review the submitted materials. The minutes will be shared with the sponsor within 30 days (U. S. Food and Drug Administration, 2017b); only after this time can studies be initiated. So, it would be ideal for sponsors to allow more than 2 months before toxicity testing. Finally, three of the four reviewers had personal experiences related to a pre-IND issue, thus

highlighting the criticality of a pre-IND consultation. Therefore, every sponsor should seriously consider reaching out to the FDA for a pre-IND consultation program, which can eventually smooth the approval of IND.

Reasons for a Low Response Rate

This study had a very low response rate of only 5%. Many reasons can be suggested for this. The FDA is an institutional body with stringent rules. Employees were strictly prohibited from accessing third party emails; hence, the researcher's FDA liaison forwarded the survey link to the personal mail IDs of interested participants. Many times employees may not have a chance or the time to respond to a survey received on their personal mail accounts.

There was no possibility for the researcher to personally meet the FDA reviewer staff. Hence, the seriousness, depth, confidentiality, and purpose of the survey could not be communicated. If this had been done, it may have slightly improved the response rate.

Based on this researcher's personal experience of working in pharmaceutical industries, it appears that FDA responds to emails of pharmaceutical submissions. Meeting in person is a challenge for companies as well because the FDA insists on having a pre-appointment booked, and only then will it respond. Being a student, the privilege of having an email account with a pharmaceutical company was nonexistent, so working for an academic institute and choosing this concept for study was the major setback that resulted in a poor response rate.

Part 2 – Review of Previously Submitted Non-Clinical Studies

In the second phase of the research, non-clinical studies submitted for synthetic peptides approved between 2010 and 2017 were evaluated for patterns in similarities and

differences. Apart from Lucinactant, a synthetic peptide containing surfactant for use in acute respiratory distress syndrome in neonates, all studied peptides had similar general safety studies or evidence submitted with their application for marketing approval. Another general observation was that no non-clinical studies were conducted for angiotensin-II because this was an exogenously administered endogenous hormone (human form) with well-known pharmacological action. Hence, upon the FDA's request, the sponsor submitted targeted literature research summaries for all non-clinical safety study categories.

The major species for conducting single dose toxicity for the included synthetic peptides was the rat, followed by mice and monkeys. Single-dose toxicity was studied in dogs for only icatibant and macimorelin. No single-dose toxicity study was conducted for etecalcitide, although no specific explanation was provided in the corresponding pharmacology review.

Repeat dose toxicity studies were also mostly conducted in rats, mice, and monkeys similar to single-dose toxicity studies. Preferred species for long-term toxicology studies of 39 or 52 weeks were mammals, including monkeys and dogs. No long-term studies conducted in rats were submitted. Since macimorelin is a single-dose administration drug for diagnosis of growth hormone deficiency, general toxicity was conducted in rats and dogs up to 4 weeks. However, for drugs with long-term administration such as anti-diabetics liraglutide and semaglutide, safety was studied for a maximum of 52 weeks in monkeys.

For all the synthetic peptides, genotoxicity was tested using the standard battery of tests including the Ames test, *in vitro*, and *in vivo* genetic damage tests. The Ames test was mainly done on strains of E.coli and S. typhi. For icatibant, pasireotide, and macimorelin, the Ames test included only S.typhi strains. *In vitro* DNA damage in mammalian cells was tested

using assay of L5178Y/TK+/- mouse cells, cultured human peripheral lymphocytes, CHO-K1 cells, or V79 cells. Most of the approved peptides included assays involving cultured human lymphocytes and L5178/TK+/- mouse cells. The more recently approved peptides, etecalcitide and macimorelin, also included aberration studies conducted in Chinese hamster ovarian (CHO-K1) cells and Chinese hamster lung fibroblast (V79) cells. An *in vivo* genotoxicity test was always conducted for the synthetic peptides (except linaclotide and macimorelin) and included either a rat or mice micronucleus test. In addition to this standard battery, *in vivo* testing was conducted for lanreotide and etecalcitide using a transgenic rodent model (Muta™Mouse). For macimorelin, genotoxicity was additionally assessed using the *in silico* prediction programs, DEREK evaluation and MCASE evaluation. As described in a 2014 review of retrospective FDA approval history starting in 1998, it may not be necessary to conduct genotoxicity testing for peptides containing natural amino acids (Sawant, Fielden, & Black, 2014); it depends entirely on the molecular composition of the synthetic peptides whether genotoxicity evaluation is required or not.

Carcinogenicity studies were submitted for all included synthetic peptides except for icatibant, carfilzomib, and macimorelin. Carcinogenicity evaluation routinely included testing for 2-year period in mice, rats, or both. In some cases (pasireotide, etecalcitide), transgenic mice were also evaluated for a 6-month duration. As carfilzomib is an anti-cancer pharmaceutical, carcinogenicity testing was not needed as per ICH S9; with the other two drugs being for intermittent (icatibant) or single (macimorelin) use, carcinogenicity evaluation was not needed. A more detailed carcinogenicity risk evaluation was conducted for semaglutide using *in vitro* thyroid C cell-lines and specific testing for calcitonin levels predictive of thyroid c-cell tumors (Guesgen et al., 2013). For liraglutide, carcinogenicity

evaluation included only mechanistic studies for thyroid cell tumors, but the recent molecule semaglutide involved more thorough evaluation. This indicates the criticality of non-clinical testing for carcinogenicity evaluation and changing requirements in relation to the advancements in the field. Thus, consultations with the FDA are crucial.

The developmental and reproductive studies for almost all peptides were conducted mostly in rodents for all listed synthetic peptides. By specific parameter of toxicity evaluation, studies analyzing effects on reproduction or fertility (segment 1) were performed in mice, rats, or both. Teratology or embryo-fetal toxicity (segment 2) and pre- and post-natal developmental toxicity (segment 3) were also evaluated in mice, rats, and sometimes rabbits. For only semaglutide, teratology and pre- and post-natal developmental studies were additionally evaluated in monkeys.

Among all the other non-specific studies conducted, testing for effects of impurities was the common category of study for six selected peptides. These tests often included general toxicological evaluation for impurities, mostly for a 4-week duration, and also genotoxicity evaluation. Apart from impurities, a series of mechanistic studies was often submitted with the applications that varied from case to case for peptides, depending on the toxicological findings from the previous non-clinical studies. For example, embryotoxicity was explored for semaglutide, mutagenicity was explored for etecalcitide, and T-cell tumorigenic potential was explored for liragutide.

Chapter 6: Conclusion

Despite having only four survey respondents, the research provided some interesting findings. Most of the CDER reviewers agreed to share informal advice prior to IND submission, but only through a pre-IND meeting, and two preferred a face-to-face meeting. Three of the reviewers agreed that the appropriate contact person for pre-IND advice is a supervisory project manager, which is not known as per the literature. In the case of synthetic peptides, pre-IND consultation is reiterated by two reviewers for the genotoxicity testing and also while having issues with species selection. The second part of the research discovered great variability for the non-clinical studies submitted with the NDAs of the recently approved synthetic peptides. Further comprehensive research on variability of each type of safety study along with route of administration, can help to understand changing regulations over time. Overall, the research highlights that regular and early interactions with the FDA will be crucial for an optimistic response from the FDA.

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APPENDICES

Appendix A: Supplementary Table

Objectives and Examples of Safety Studies

Type of Study and Description
Acute Toxicity • To determine preliminary safety of a new molecule by observing the nature and duration of any adverse events • To find out short term adverse effects on the administration of the study drug in a single dose, or in multiple doses during 24 hours in two mammalian species (one nonrodent) through the clinical route of administration and find the maximum tolerated dose • Studies involve steadily increasing the dose (single or a number of consecutive doses), until adverse effects (such as vomiting and convulsions) indicating that an maximum tolerated dose has been reached • Animals are typically observed for 14 days *Examples:* Fixed Dose Procedure (OECDTG 420) AcuteToxic Class method (OECDTG 423) Up-and-Down Procedure (OECDTG 425) Acute DermalToxicity (OECDTG 402) Acute inhalation toxicity
Sub-acute/Sub-chronic Toxicity • To identify adverse effects that develop over a period of continuous exposure to the new drug (28 days – sub acute; 90 days - subchronic) • To identify the most affected organs and determine the doses at which each effect occurs • Typically, of 28 days to 90 days duration, done in rats and mice *Examples* Repeated Dose 28-day Oral Toxicity Study in Rodents (TG407) Repeated Dose 90-Day Oral Toxicity Study in Rodents (TG 408) Repeated Dose Dermal Toxicity: 21/28-day Study (TG 410) Subchronic Dermal Toxicity: 90-day Study (TG 411) Repeated Dose Inhalation Toxicity: 28-day or 14-day Study (TG 412) Subchronic Inhalation Toxicity: 90-day Study (TG 413)

Table *continued*

Type of Study and Description
Chronic Toxicity • To examine the cardiovascular, respiratory and CNS effects of the study drug. Various specific tests include: *Cardiovascular* • Dog telemetry • hERG assay • isolated Purkinje nerve fibers assays *Respiratory* • Evaluation of the "respiratory pump" efficiency and gas exchange • Whole body plethysmography - to evaluate parameters including tidal volume, minute volume and mid-expiratory flow (EF50) *CNS* • To observed compound effects on general behavior, locomotion, neuromuscular coordination, seizure threshold, and vigilance through functional observation battery and Irwin test
Genotoxicity • These tests identify compounds that can induce genetic damage • These are mostly required for small molecules, but not generally required for biologics Standard battery of tests include: • Bacterial reverse gene mutation test • *In vitro* chromosomal aberrations using Ames test • In *in vitro* mammalian cells using the following: ○ *In vitro* metaphase chromosome aberration assay ○ *In vitro* micronucleus assay ○ mouse lymphoma L5178Y cell Tk gene mutation assay • In *in vivo* test using rodent hematopoietic cells, either for micronuclei or for chromosomal aberrations in metaphase cells

Table *continued*

Type of Study and Description
Developmental and Reproductive Toxicity • *Teratogenecity* Embryonic and fetal tests are usually performed in two or three species (rats, mice, rabbits) by administering drug to females in the initial period of pregnancy (in rats, 6–16 days after mating). • *Male and Female fertility* Fertility and implantation tests include male (28 days) and female (14 days) treatments with the substance before mating, and are characterized by the semen analysis (counting and viability), number of implanted embryos and survival of the embryos at the sixth day of pregnancy • *Pre-/post-natal development* In these tests, females are treated during pregnancy and lactation. Post-lactation motor activity, any abnormalities in different stages of development, their sexual performance and second offspring among the pups are studied
Carcinogenicity • To assess the risk of cancer induction by the chemical in exposed humans • Incidence and type of the tumors that develop in rats and mice when dosed for up to two years (the typical lifespan)

Note. CNS, central nervous system; OECDTG, Organisation for Economic Co-operation and Development Test Guidelines; hERG, human ether-a-go-go-related gene.

From "Non-clinical studies in the process of new drug development: Part II: Good laboratory practice, metabolism, pharmacokinetics, safety and dose translation to clinical studies" by E. L. Andrade, A. F. Bento, J.Cavalli, S. K. Oliveira, R. C. Schwanke, J. M. Siqueira, C. S. Freitas, R. Marcon, and J. B. Calixto, 2016, *Brazilian Journal of Medical and Biological Research, 49*, p7–17;

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Appendix B: Questionnaire

1)		**Position at FDA**
	a.	Reviewing Pharmacologist
	b.	Supervisory Pharmacologist
	c.	Other
2)		**Review Center**
	a.	CDER
	b.	CBER
	c.	Other
3)		**Years at FDA**
	a.	0 to 2
	b.	2 to 5
	c.	6 to 10
	d.	Greater than 10
4)		**Are you willing/able to provide informal advice to sponsors prior to submission of an IND**
	a.	Yes
	b.	Yes, but time is extremely limited
	c.	Yes, but a pre-IND meeting request and supporting documentation must be submitted
	d.	No, an active IND must be on file
5)		**The best way to obtain advice prior to IND submission is via**
	a.	Teleconference to discuss previously submitted materials
	b.	Face-to-face meeting to discuss previously submitted materials
	c.	Email replies from a project manager to very specific questions
	d.	Email replies from a reviewer to very specific questions

6)		**The appropriate contact to request pre-IND advice is**
	a.	The supervisory project manager
	b.	Any divisional project manager
	c.	The division director
	d.	Directly to a reviewing or supervisory pharmacologist
7)		**Synthetic peptides should be considered, for genotoxicity purposes,**
	a.	To be handled like small molecules
	b.	To be handled like a biologic product
	c.	To vary by compound. Pre-testing consultation should be sought.
	d.	Unknown
8)		**If human synthetic peptides or biologics do not show the same pharmacology in animal models as they do in human tissue cultures, it this a concern for selection of species for toxicology testing?**
	a.	No. Rodent and non-rodent studies should be conducted as usual
	b.	No, as long as dose-limiting toxicity is above the expected human therapeutic dose
	c.	Yes, the animal model used for toxicity testing must exhibit the same pharmacology as that expected in humans
	d.	Pre-testing consultation with the Review Division should be sought.
9)		**If pre-testing consultation is required before beginning toxicology tests, how much time should the sponsor build into their planning to allow appropriate input from the agency?**
	a.	1 to 2 weeks
	b.	1 month
	c.	2 months
	d.	More than 2 months
10)		**Have you had personal experience dealing with issues like those mentioned in this survey.**
	a.	Yes for issues pre-IND, but not those specific ones mentioned here.

	b.	Yes
	c.	No

Appendix C: UHSRC Approval letter

RESEARCH @ EMU

UHSRC Determination: **EXEMPT**

DATE: **April 13, 2016**

TO: **Vamsi Vardhan Niraghatam**
School of Health Sciences
Eastern Michigan University

Re: **UHSRC: # 878601-1**
Category: Exempt category 2
Approval Date: April 13, 2016

Title: **Perceptions of FDA towards pre-clinical studies**

Your research project, entitled **Perceptions of FDA towards pre-clinical studies,** has been determined **Exempt** in accordance with federal regulation 45 CFR 46.102. UHSRC policy states that you, as the Principal Investigator, are responsible for protecting the rights and welfare of your research subjects and conducting your research as described in your protocol.

Renewals: Exempt protocols do not need to be renewed. When the project is completed, please submit the **Human Subjects Study Completion Form** (access through IRBNet on the UHSRC website).

Modifications: You may make minor changes (e.g., study staff changes, sample size changes, contact information changes, etc.) without submitting for review. However, if you plan to make changes that alter study design or any study instruments, you must submit a **Human Subjects Approval Request Form** and obtain approval prior to implementation. The form is available through IRBNet on the UHSRC website.

Problems: All major deviations from the reviewed protocol, unanticipated problems, adverse events, subject complaints, or other problems that may increase the risk to human subjects **or** change the category of review must be reported to the UHSRC via an **Event Report** form, available through IRBNet on the UHSRC website

Follow-up: If your Exempt project is not completed and closed after **three years**, the UHSRC office will contact you regarding the status of the project.

Please use the UHSRC number listed above on any forms submitted that relate to this project, or on any correspondence with the UHSRC office.

Good luck in your research. If we can be of further assistance, please contact us at 734-487-3090 or via e-mail at human.subjects@emich.edu. Thank you for your cooperation.

Sincerely,

Heather Hutchins-Wiese
Chair
CHHS Human Subjects Review Committee