Scientific and Regulatory Considerations for the Approval of the First Generic Glucagon

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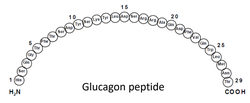
Introduction

The development of generics for peptide drug products requires some additional considerations compared to non-peptide drugs. To support the development of safe and effective generic versions of peptide drug products, the FDA published its current thinking on how to ensure sameness between the generic and innovator peptide products, aka reference listed drugs (RLD), prepared with different manufacturing processes in a draft guidance for industry titled "ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin", which applies to five peptide drug products, including glucagon [1].

Glucagon for Injection (NDA 020928) is a polypeptide hormone identical to human glucagon approved over 20 years ago for the treatment of severe hypoglycemia. Despite glucagon for injection's long history, there had been no generic versions approved. Recently on Dec 28, 2020, the U.S. FDA approved the first generic version of glucagon for injection USP, 1 mg/vial packaged in an emergency kit. The generic and the RLD version of glucagon are each produced through different manufacturing processes. The RLD version of glucagon is produced via recombinant DNA (rDNA) in yeast while the generic version of glucagon is produced by peptide synthesis. To support the sameness of the generic product to the RLD the applicant analyzed, compared, and controlled the types and amounts of impurities in the generic product. In addition, the potential immunogenicity of new impurities, which are not in the RLD product, were assessed and compared using non-clinical assays. Here we will discuss various non-clinical assays that may be used for assessing the immunogenicity risk of these impurities, for both adaptive and innate immune responses.



Glucagon peptide

Glucagon injection Amphastar

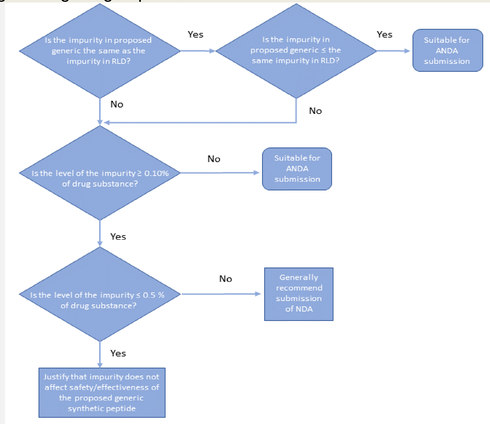
Guidance Recommendations

The first generic synthetic glucagon was approved following the recommendations outlined in the draft guidance for industry titled "ANDAs for Certain Highly Purified Synthetic Peptide Drug Products That Refer to Listed Drugs of rDNA Origin". To demonstrate active pharmaceutical ingredient (API) sameness, analytical methods and other laboratory techniques may be used to evaluate primary and secondary structures, oligomers and aggregation states, and compare physicochemical properties. Assays of biological activity may also be used to demonstrate API sameness. Demonstrating API sameness between generic and RLD of peptide drug products using analytical and biological methods is a routine part of generic drug development, and therefore will not be discussed further.

Whether a peptide is produced by a recombinant or synthetic process, impurities may result from the insertion, deletion, or modification of amino acid sequences or residues. These impurities generally pose minimal safety or efficacy risks and levels of these impurities can be controlled. However, in some circumstances, peptide-related impurities may create the potential for differences in immunogenicity. The aforementioned guidance focuses on providing recommendations on evaluating peptide related impurities (e.g., those impurities related to the synthesis or degradation of the peptide), and summarized (Figure 1) as follows: 1) show that, for each peptide-related impurity that is found in both the proposed generic synthetic peptide and the RLD, the level of such impurity in the proposed generic synthetic peptide is the same as or lower than that found in the RLD; 2) show that the proposed generic synthetic peptide does not contain any new specified peptide-related impurity that is more than 0.5 percent of the drug substance; 3) characterize each new specified peptide related impurity; and 4) justify for each new specified peptide-related impurity that is no more than 0.5% of the drug substance why such impurity does not affect the safety of the proposed generic synthetic peptide and does not affect its effectiveness. This assessment may include using non-clinical methods to ensure the risk of immunogenicity due to peptide-related impurities will not differ from that of the RLD of rDNA origin. It should be noted that clinical studies to provide safety or effectiveness data are not part of a generic drug product application as generics must rely on the clinical safety and efficacy profiles and findings of the RLD.

To demonstrate that the risk of immunogenicity in the generic peptide is no greater than the RLD's, the guidance recommends comparing the innate immune response and adaptive immune response in the generic and the RLD products using non-clinical methods (i.e. in silico, in vitro, and in vivo animal). For assessing the effect on adaptive immune response, it is recommended that the applicant demonstrate that each new impurity does not contain sequences that have an increased affinity for binding to T-cell epitopes as compared to the API. For innate immune response, the applicant should provide data showing that the generic peptide product does not alter the innate immune activity in comparison to the RLD's.

Figure 1. Flow chart for evaluating each impurity in a proposed generic glucagon product



Non-clinical Immunogenicity Assessments and Discussion

Various immune cell-based assays have been demonstrated to correlate with the rate of clinical immunogenicity through combinations of immune cells, broad HLA genotypes, multiple assay readouts, etc. Here, we will discuss some of these non-clinical immunogenicity assessment assays an applicant can use to demonstrate the immunogenicity risk of a proposed generic peptide to be no greater than that of the RLD.

When assessing the innate immune responses, a number of in vitro cell-based methods, such as cell-based methods using macrophage cell lines, monocyte-derived dendritic cells (DCs), and peripheral blood mononuclear cells (PBMCs) can be used to assess this risk (examples of these assays can be found in Table 1). The developer of a generic peptide can choose any of these in vitro methods to evaluate the innate immune response of their product in comparison that of the RLD's. All of the in vitro cell based methods utilizes cells that can be commercially purchased. Some of these methods, such as using PBMCs and monocyte-derived DCs, require obtaining blood from a donor population. The working principles behind of many of these assays are similar, in that the cytokine release readouts are measured as the test drugs are incubated with cells responsible for innate immune activation. To be considered for approval, the generic peptide product should trigger a similar or lower amount of cytokine release in comparison to the RLD.

To assess the adaptive immune responses of any specific peptide-related impurities, the applicant can assess if the new impurity contains sequences that can increase affinity for binding to T-cell epitopes. There are commercially available in silico tools to assess and predict the T-cell epitope binding in populations with specific HLAs. In addition, there are various in vitro cell-based methods for assessing adaptive immune response (for examples, see Table 2.) The in vitro assays, such as PBMC assays, DC-T cell assays, and T cell-PBMC assays, are all commercially available. However, in order to properly and sufficiently assess the risk, the assay must use blood from a sufficient number of donors to get a meaningful result. In order to correctly conduct these in vitro assays, assay parameters including sensitivity and specificity, test product concentrations, cell viability or metabolic activity, positive standard selection and excipient effects, need to be controlled and validated.

Table 1. Examples of In Vitro Cell Based Methods to Assess Innate Immune Response

Assay	Cells	Commercially available	Donor needed	Readouts
IIRMIs cell based assay [2]	Macrophage cell lines (RAW-BLUE, MM6)	Y	N	RAW-BLUE: SEAP reporter construct inducible by NF-κB. MM6: mRNA levels of IL-6 and IL-8
Innate immune response to stress-induced aggregates [3]	Human monocyte-like cell lines THP-1 and MM6.	Y	N	The concentrations of inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, IL-10 and IL-12)
In vitro assay to monitor DC maturation [4]	Monocyte-derived DCs	Y	Y	DC activation markers and cytokine release
PBMC Stimulation Assay [5]	PBMC	Y	Y	Cytokine analysis (The cytokines include IL-1β, IL-6, IL-10, MCP-1, MIP-1α, MIP-1β, MMP-2, and TNF-α.)
Peripheral Tissue Equivalent (PTE) module [6]	A three-dimensional endothelial cell / collagen matrix culture system, co-cultured with PBMC.	Y	N	Production of cytokines and chemokines, such as interleukins 1a/b, 6, 8 and 10 and TNF-α.

Table 2. Examples of In Vitro Cell Based Methods to Assess Adaptive Immune Response

Assay	Cells	HLA genotyped	Donor needed	Readouts
IVCIA assay [7]	PBMC without CD8+ T cells	Y	Y	T cell proliferation, IL-2 concentration & secreting cells
DC-T cell assay [8]	monocyte-derived dendritic cells and CD4+ T cells	Y	Y	CD4+ T-cell proliferation, IL-2 secretion via ELISpot assay
In vitro PBMC derived T cell assay [9]	PBMC from naïve healthy donors	Y	Y	IFNg secretion via ELISpot assay.
In vitro T cell: PBMC assay [10]	purified CD4+ T cells co-cultured with irradiated PBMCs (1:2)	Y	Y	T cell proliferation and IL-2 secretion.
HLA -DR-Specific Binding Assays [11]	N/A	N	N	Data were reported as relative affinity corresponding to the ratio of the IC50 of the tested peptide to the IC50 of the reference peptide.

Conclusion

While peptides like glucagon may be produced using different manufacturing processes, the sameness of a generic peptide compared to the RLD can be adequately demonstrated using various non-clinical analytical methods. Biological assays may also be used as part of the demonstration of active pharmaceutical ingredient sameness. In addition to API sameness, generic peptide drugs also have to sufficiently demonstrate that they carry no greater immunogenicity risk than the RLD product using non-clinical assays. Both the innate and adaptive immune responses are assessed using a combination of relevant non-clinical assays. While the recent glucagon approval is the first generic peptide product approved based on the recommendations of the draft peptide guidance, the recommendations can also be applied to support the U.S. approval of other generic peptide products prescribed by endocrinologists, such as teriparatide and liraglutide, which supports greater patient access to safe and effective generic peptide drug products.

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