

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

214231Orig1s000

OTHER REVIEW(S)

MEMORANDUM
REVIEW OF REVISED LABEL AND LABELING
Division of Medication Error Prevention and Analysis (DMEPA)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

Date of This Memorandum: March 5, 2021
Requesting Office or Division: Division of Diabetes, Lipid Disorders, and Obesity (DDLO)
Application Type and Number: NDA 214231
Product Name and Strength: Zegalogue (dasiglucagon) injection, 0.6 mg/0.6 mL
Applicant/Sponsor Name: Zealand Pharma A/S
OSE RCM #: 2020-614-4
DMEPA Safety Evaluator: Colleen Little, PharmD
DMEPA Team Leader: Lolita White, PharmD

1 PURPOSE OF MEMORANDUM

The Applicant submitted revised container labels and carton labeling received on March 4, 2021 for Zegalogue. Division of Diabetes, Lipid Disorders, and Obesity (DDLO) requested that we review the revised container labels and carton labeling for Zegalogue (Appendix A) to determine if they are acceptable from a medication error perspective. The revisions are in response to recommendations that we made during a previous label and labeling review.^a

2 CONCLUSION

The Applicant implemented all of our recommendations and we have no additional recommendations at this time.

^a Little, C. Label and Labeling Review for Zegalogue (NDA 214231). Silver Spring (MD): FDA, CDER, OSE, DMEPA (US); 2021 FEB 24. RCM No.: 2020-614-3.

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/s/

COLLEEN L LITTLE
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03/07/2021 02:43:16 PM

MEMORANDUM
REVIEW OF REVISED LABEL AND LABELING
Division of Medication Error Prevention and Analysis (DMEPA)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

Date of This Memorandum: February 24, 2021
Requesting Office or Division: Division of Diabetes, Lipid Disorders, and Obesity (DDLO)
Application Type and Number: NDA 214231
Product Name and Strength: Zegalogue (dasiglucagon) injection, 0.6 mg/0.6 mL
Applicant/Sponsor Name: Zealand Pharma A/S
OSE RCM #: 2020-614-3
DMEPA Safety Evaluator: Colleen Little, PharmD
DMEPA Team Leader: Lolita White, PharmD

1 PURPOSE OF MEMORANDUM

The Applicant submitted revised container labels and carton labeling received on February 12, 2021 for Zegalogue. Division of Diabetes, Lipid Disorders, and Obesity (DDLO) requested that we review the revised container labels and carton labeling for Zegalogue (Appendix A) to determine if it is acceptable from a medication error perspective. The revisions are in response to recommendations that we made during a previous label and labeling review.^a

2 CONCLUSION

The revised carton labeling are unacceptable from a medication error perspective because they are inconsistent with the prescribing information and not in accordance with 21 CFR 201.55.

3 RECOMMENDATIONS FOR ZEALAND PHARMA A/S

We recommend the following be implemented prior to approval of this NDA:

A. Autoinjector and Prefilled Syringe Carton Labeling (1 pack and 2 pack)

1. The term [REDACTED]^{(b) (4)} is inconsistent with the prescribing information. We are concerned that use of inconsistent terminology may lead to confusion. Revise the term [REDACTED]^{(b) (4)} to "prescribing information."

^a Little, C. Label and Labeling Review for Zegalogue (NDA 214231). Silver Spring (MD): FDA, CDER, OSE, DMEPA (US); 2021 FEB 9. RCM No.: 2020-614-2.

2. The "Usual Dose" statement is not included. Per 21 CFR 201.55, "...labels for prescription drugs bear a statement of the recommended or usual dosage."
Revise the statement [REDACTED] ^{(b) (4)} similar to "Recommended Dosage: See prescribing information."

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COLLEEN L LITTLE
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02/25/2021 02:17:25 PM

**FOOD AND DRUG ADMINISTRATION
Center for Drug Evaluation and Research
Office of Prescription Drug Promotion**

*****Pre-decisional Agency Information*****

Memorandum

Date: February 24, 2021

To: Elizabeth Godwin, Regulatory Project Manager
Division of Diabetes, Lipid Disorders, and Obesity (DDLO)

Monika Houstoun, Associate Director for Labeling, (DDLO)

From: Samantha Bryant, Regulatory Review Officer
Office of Prescription Drug Promotion (OPDP)

CC: Matthew Falter, Acting Team Leader, OPDP

Subject: OPDP Labeling Comments for ZEGALOGUE (dasiglucagon) injection, for subcutaneous use

NDA: 214231

In response to DDLO consult request dated March 31, 2020, OPDP has reviewed the proposed product labeling (PI), patient package insert (PPI), Instructions for Use (IFU), and carton and container labeling for the original NDA submission for ZEGALOGUE. After further discussions with DDLO, CDRH, and DMEPA and upon further consideration and clarification regarding the descriptor, OPDP would like to update our labeling review consult response submitted on January 15, 2021 to include an additional comment regarding the use of the descriptor, (b) (4)

OPDP Internal Comment to DDLO: We are concerned that use of the descriptor, (b) (4)

(b) (4). OPDP defers to DDLO for whether the use of the descriptor, (b) (4) is appropriate for use throughout the proposed labeling for the product.

Thank you for your consult. If you have any questions, please contact Samantha Bryant at (301) 348-1711 or Samantha.Bryant@fda.hhs.gov.

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/s/

SAMANTHA E BRYANT
02/24/2021 02:30:40 PM

MEMORANDUM
REVIEW OF REVISED LABEL AND LABELING
Division of Medication Error Prevention and Analysis (DMEPA)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

Date of This Memorandum: February 9, 2021
Requesting Office or Division: Division of Diabetes, Lipid Disorders, and Obesity (DDLO)
Application Type and Number: NDA 214231
Product Name and Strength: Zegalogue (dasiglucagon) injection, 0.6 mg/0.6 mL
Applicant/Sponsor Name: Zealand Pharma A/S
OSE RCM #: 2020-614-2
DMEPA Safety Evaluator: Colleen Little, PharmD
DMEPA Team Leader: Lolita White, PharmD

1 PURPOSE OF MEMORANDUM

The Applicant submitted revised container labels and carton labeling received on February 1, 2021 for Zegalogue. Division of Diabetes, Lipid Disorders, and Obesity (DDLO) requested that we review the revised container labels and carton labeling for Zegalogue (Appendix A) to determine if they are acceptable from a medication error perspective. The revisions are in response to recommendations that we made during a previous label and labeling review.^a

2 CONCLUSION

The revised protective case labeling for the autoinjector (AI) and prefilled syringe (PFS) presentations are unacceptable from a medication error perspective. The PFS protective case labeling includes inconsistent terminology regarding the expiration date. Additionally, the AI protective case label includes similar images of opening the protective case in different positions (i.e., horizontal and vertical), which may lead to confusion. We provide recommendations to address these concerns in Section 3 below.

3 RECOMMENDATIONS FOR ZEALAND PHARMA A/S

^a Little, C. Label and Labeling Review for Zegalogue (NDA 214231). Silver Spring (MD): FDA, CDER, OSE, DMEPA (US); 2021 JAN 21. RCM No.: 2020-614-1.

We recommend the following be implemented prior to approval of this NDA:

A. Autoinjector (AI) Protective Case labeling

1. The term (b) (4) is inconsistent with the Instructions for Use (IFU). For example, the IFU includes the statement "Do not use Zegalogue if the expiration date has passed." We are concerned that use of inconsistent terminology may lead to confusion. Revise the term (b) (4) to "Expiration date" or "EXP date" on the AI protective case labeling.

B. Prefilled Syringe (PFS) Protective Case labeling

1. We acknowledge that you have included an image of opening the protective case on the 'middle' panel in the vertical position of the PFS protective case labeling. We have concerns with your proposal to still retain the original image of opening the protective case in the horizontal position. We find that the inclusion of similar images in different positions (i.e., horizontal vs. vertical) may lead to confusion regarding the correct orientation of the protective case while opening. We note that the AI protective case includes a single image of opening the protective case in the vertical position and no failures or confusion were seen in the human factors study for the open protective case task. Thus, we recommend that you remove the horizontal image (b) (4) (b) (4) of the protective case labeling and retain the vertical image on the 'middle' panel to decrease risk of confusion.

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COLLEEN L LITTLE
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LOLITA G WHITE
02/09/2021 04:15:37 PM



DIVISION OF DRUG DELIVERY, GENERAL HOSPITAL & HUMAN FACTORS
INTERCENTER CONSULT MEMORANDUM

Date	4/30/2020		
To:	Leeza Rahimi, Sr. Regulatory Health Project Manager		
Requesting Center/Office:	CDER/OPQ	Clinical Review Division:	DMEP
From	David Wolloscheck, PhD, Chemist OPEQ/OHT3/DHT3C		
Through (Team)	Courtney Evans, Acting Team Lead, Injection Team OPEQ/OHT3/DHT3C		
Through (Division) *Optional	Rumi Young, Acting Assistant Director OPEQ/OHT3/DHT3C		
Subject	NDA214231 , Dasiglucagon Injection ICC2000299 00018601/00018306		
Recommendation	Filing Recommendation Date: 5/13/2020 ☐ CDRH did not provide a Filing Recommendation ☐ Device Constituent Parts of the Combination Product are acceptable for Filing. ☒ Device Constituents Parts of the Combination Product are Acceptable for Filing with Information requests for the 74-Day Letter, See Appendix A ☐ Device Constituents Parts of the Combination Product are Not Acceptable for Filing - See Section 5.4 for Deficiencies We are recommending inspection for the following facilities: (b) (4) Rechon Life Science AB (FEI: 3002806978)		
	Mid-Cycle Recommendation Date: 8/11/2020 ☐ CDRH did not provide a Mid-Cycle Recommendation ☐ CDRH has no approvability issues at this time. ☒ CDRH has additional Information Requests, See Appendix A ☐ CDRH has Major Deficiencies that may present an approvability issue, See Appendix A.		
	Final Recommendation Date: 1/26/2021 ☒ Device Constituent Parts of the Combination Product are Approvable. ☐ Device Constituent Parts of the Combination Product are Approvable with Post-Market Requirements/Commitments, See Section 2.3 ☐ Device Constituent Parts of the Combination Product are Not Approvable - See Section 2.2 for Complete Response Deficiencies We recommend a Post-Approval Inspection of the following manufacturing site: Rechon Life Science AB (FEI: 3002806978)		

Digital Signature Concurrence Table		
Reviewer	Team Lead (TL)	Division (*Optional)

1. SUBMISSION OVERVIEW

Submission Information	
Submission Number	NDA214231
Sponsor	Zealand Pharma A/S
Drug/Biologic	Dasiglucagon Injection
Indications for Use	Hypoglycemia in patients with diabetes aged 6 years and above.
Device Constituent	Auto-Injector
Related Files	ICC1900759

Review Team		
Lead Device Reviewer	<i>David Wolloscheck, PhD, Chemist</i>	
Discipline Specific Consults	Reviewer Name (Center/Office/Division/Branch)	CON #
N/A		

Important Dates	
Final Lead Device Review Memo Due	2/19/2021 (PDUFA: 3/26/2021)
Interim Due Dates	
	Meeting/Due Date
Filing	5/7/2020 (OND); 5/26/2020 (CMC)
74-Day Letter	5/26/2020
Mid-Cycle	7/16/2020 (CMC); 7/30/2020 (OND)
Primary Review	11/13/2020 (CMC); 11/27/2020 (OND)
Sponsor Meeting(s)	09/14/2020 (TCON w/ Applicant)

2. EXECUTIVE SUMMARY AND RECOMMENDATION

CDRH recommends the combination product is:

- Approvable – the device constituent of the combination product is approvable for the proposed indication.
- Approvable with PMC or PMR, [See Section 2.3](#)
 - Not Acceptable – the device constituent of the combination product is not approvable for the proposed indication. We have Major Deficiencies to convey, [see Section 2.2](#).

Section	Adequate			Reviewer <u>Notes</u>
	Yes	No	NA	
Device Description	X			
Labeling	X			
Design Controls	X			
Risk Analysis	X			
Design Verification	X			
Consultant Discipline Reviews			X	
Clinical Validation	X			
Human Factors Validation			X	Deferred to DMEPA
Facilities & Quality Systems	X			The 704a facilities desk review has concluded. Recommend post-approval inspection to evaluate final, commercial auto-injector assembly line validation.

2.1. **Comments to the Review Team**

- CDRH does not have any further comments to convey to the review team.
- CDRH has the following comments to convey to the review team:

Comment #1: The final commercial Auto-Injector assembly has not been fully validated. Two modifications were made to the assembly line after it was validated for the manufacturing of clinical batches of the AI. (b) (4)
(b) (4). As this is a high risk emergency use injector, we recommend a post-approval inspection to evaluate the final commercial AI assembly validation.

2.2. **Complete Response Deficiencies**

- There are no outstanding unresolved information requests, therefore CDRH does not have any outstanding deficiencies.
- The following outstanding unresolved information requests should be communicated to the Sponsor as part of the CR Letter:
 No CR deficiencies were identified. However, stability and facilities review is ongoing.

2.3. **Recommended Post-Market Commitments/Requirements**

CDRH has Post-Market Commitments or Requirements	☐
CDRH does not have Post-Market Commitments or Requirements	☒

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3. PURPOSE/BACKGROUND

3.1. Scope

Zealand Pharma A/S is requesting approval of Dasiglucagon Injection. The device constituent of the combination product is a Choose an item..

CDER/OPQ has requested the following [consult](#) for review of the device constituent of the combination product:

- Review of device (engineering) discussed in emails
- Review of facilities

The goal of this memo is to provide a recommendation of the approvability of the device constituent of the combination product. This review will cover the following [review areas](#):

- Device Performance
- Device Reliability
- Biocompatibility
- Facilities

This review will not cover the following review areas:

- Human Factors

The original review division will be responsible for the decision regarding the overall safety and effectiveness for approvability of the combination product.

3.2. Prior Interactions

CDRH has issued comments to the Sponsor regarding a marketing application for Dasiglucagon four times. The Sponsor was issued comments related to the device verification and validation activities, documentation to submit in the NDA, and comments related to the reliability requirements for the syringe and autoinjector. The Sponsor provided a fault tree analysis for the Prefilled syringe (PFS) and Autoinjector (AI) presentations which were found acceptable by the reviewer. Zealand is proposed a reliability specification of 99.99% for the PFS and the following reliability specifications for the AI:

Reliability Requirement	Reliability Specification / Confidence Level
Successful Activation	NLT 99.999% / 95%
Dose Accuracy	99.99% / 95%
Extended Needle Length	99.99% / 95%
Activation Force	99.99% / 95%
Injection Time	99.99% / 95%

This meeting request does not specifically discuss the reliability requirements of the device constituent parts.

The Agency further interacted with the Sponsor to clarify the stability data requirements for the initial NDA submission and the structure of the to-be-submitted information.

3.2.1. Related Files

3.3. Indications for Use

Combination Product	Indications for Use
Dasiglucagon Injection	Hypoglycemia in patients with diabetes aged 6 years and above.
Auto-Injector and Pre-filled Syringe	Delivery of the Drug Product

3.4. Materials Reviewed

Materials Reviewed	
Sequence	Module(s)
0001	M3: 3.2.P.7
0001	M3: 3.2.R

4. DEVICE DESCRIPTION

4.1. Device Description

Dasiglucagon is intended for the emergency treatment of hypoglycemia. The proposed combination product will come in a pre-filled safety syringe (b) (4) and an autoinjector. The autoinjector is loaded with the same pre-filled syringe (b) (4). The following high level overview of the manufacturing was provided:



Pre-filled Syringe Component (PFS)

The following diagram highlights the components that are identical for both the final Pre-filled syringe (PFSS) and Auto-Injector (AI): (b) (4)



(b) (4)

The components of the prefilled syringe (PFS) are manufactured by

(b) (4)

(b) (4) The following table was provided highlighting the component manufactures:

Manufacturer	Site Address	FDA establishment identifier (FEI)	DMF / 510K number	Responsibilities

(b) (4)



Pre-filled Syringe with Rigid Needle Shield (PFSS)

The proposed PFSS consists of a rigid needle shield (RNS), (b) (4) the drug product, the 1 mL glass syringe with prestaked 27Gx12.7mm needle, and the plunger rod with stopper. The following image was provided in the submission:



Zealand provided the following table describing the materials and components used in the PFSS:

<i>Part</i>	<i>Manufacturer</i>	<i>Component</i>	<i>Grade/Type</i>	<i>Function</i>	<i>Conformity</i>
Syringe with staked needle					

(b) (4)



The following engineering drawings with dimensions of the primary container closure were provided:



The syringe will be packaged in the following protective case to secure the device while the patient is carrying it:



Auto-Injector (AI)

The proposed autoinjector includes the same primary container closure for the drug product solution as the PFSS. The injector consists of a yellow needle guard, a cap, the prefilled syringe, and a front and rear sub-assembly. The sub-
(b) (4) are manufactured by (b) (4) for which the manufacture has a master file (b) (4). The device contains a medication window. The following images were provided:



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The following image and component information was provided by Zealand for the front sub-assembly of the device:



Table 4 Components of front sub-assembly

No.	Component	Raw material	Color	
1	Front cap (Cap)	(b) (4)		
2	Front cap cover			
3	RNS remover			
4	(b) (4)			
5	Needle cover (Needle guard)			

		(b) (4)
6	Body	
7	Syringe housing	

Rear Sub-Assembly

The Sponsor provided the following information regarding the rear sub-assembly of the AI and component materials:



Table 6 Components of rear sub-assembly

No.	Component	Raw material	Color	Function
1	Plunger rod	(b) (4)		
2				
3				
4				

(b) (4)

The AI is packaged in the following protective casing  (b) (4) :

(b) (4)

Letters of Authorization

The Sponsor provided letters of Authorization for the following files:

-  (b) (4)
-
-
-
-

4.2. Steps for Using the Device

Pre-filled Syringe

1. The user removes the needle cover
2. Pinch the skin and insert the needle at a 45° angle
3. Give injection by depressing the plunger
4. Once plunger rod stops, remove needle from injection site

(b) (4)



Auto-Injector

1. Remove the cap from needle end
2. Push the injector straight down onto the injection site. You may hear a first click.
3. Hold the injector down and count to 10. The medicine window turns red and you may hear a second click.
4. Confirm that the medicine window is red and remove the injector by lifting straight up. Yellow needle guard will cover the needle.





4.3. Device Description Conclusion

DEVICE DESCRIPTION REVIEW CONCLUSION		
Filing Deficiencies:	Mid-Cycle Deficiencies:	Final Deficiencies:
☐ Yes ☒ No ☐ N/A	☐ Yes ☒ No ☐ N/A	☐ Yes ☒ No ☐ N/A
Reviewer Comments The Sponsor provided a full description of the device with engineering drawings and dimensions. No further deficiencies were identified.		
CDRH sent Device Description Deficiencies or Interactive Review Questions to the Sponsor: ☐ Yes ☒ No		

5. FILING REVIEW

CDRH performed Filing Review	☒
CDRH was not consulted prior to the Filing Date; therefore CDRH did not perform a Filing Review	☐

5.1. Filing Review Checklist

Filing Review Checklist				
Description	Present			
	Yes	No	N/A	
Description of Device Constituent	X			
Device Constituent Labeling	X			
Letters of Authorization	X			
Essential Performance Requirements defined by the application Sponsor	X			
Design Requirements Specifications included in the NDA / BLA by the application Sponsor	X			
Design Verification Data included in the NDA / BLA or adequately cross-referenced to a master file.	X			
Risk Analysis supplied in the NDA / BLA by the application Sponsor	X			
Traceability between Design Requirements, Risk Control Measures and V&V Activities	X			
Verification/ Validation Check	Full Test Reports for Verification and Validation Testing		X	
	Engineering Performance (must include Safety Assurance Case for Infusion Pumps)		X	
	Reliability	X		
	Biocompatibility	X		
	Sterility			X
	Software			X
	Cybersecurity			X
	Electrical Safety			X
	EMC/RF Wireless			X
	MR Compatibility			X
	Human Factors	X		
	Shelf Life, Aging and Transportation	X		
	Clinical Validation	X		
	Human Factors Validation	X		
Quality Systems/ Manufacturing Controls Check	Description of Device Manufacturing Process	X		
	Description of Quality Systems (Drug cGMP-based, Device QSR-based, Both)	X		
	CAPA Procedure	X		
	Control Strategy provided for EPRs	X		

Reviewer Comment

Zealand has provided summary reports of the verification activities for both the PFSS and AI. However, full test reports were not provided. An IR will be recommended for the 74-Day letter.

5.2. Facilities Information

Firm Name:	Rechon Life Science AB
Address:	<u>Soldatorpsvaegen 5, 216 13 Limhamn, Sweden</u>
FEI:	3002806978
Responsibilities:	Storage of drug substance.

	• Constituent parts at the site: drug and device. • Drug product manufacturing, including filling into pre-filled syringes, visual inspection, final assembly into prefilled safety syringe and auto-injector, labelling and packaging. • Release and stability testing of drug product. • Warehousing of primary and secondary packaging material, filled pre-filled syringes and final assembled pre-filled safety syringes and autoinjectors.
<u>Inspectional History</u> An analysis of the firm's inspection history over the past 2 years: ☒ Inspection was conducted (b)(4) The inspection covered drug CGMP and was classified VAI. ☐ An analysis of the firm's inspection history over the past 2 years showed that it has not been inspected. ☐ N/A - the manufacturing site does not require an inspection at this time given the risk of the combination product	
<u>Inspection Recommendation:</u> ☒ A pre-approval inspection <u>is required</u> because: The firm is responsible for major activities related to the manufacturing and/or development of the final combination involving the device constituent part; and, A recent medical device inspection of the firm <u>has not been performed</u>. ☐ An inspection <u>is not required</u> because Choose an item.	

Firm Name:		(b)(4)
Address:		
FEI:		
Responsibilities:		
<u>Inspectional History</u> An analysis of the firm's inspection history over the past 2 years: ☐ Inspection was conducted Click or tap to enter a date. to Click or tap to enter a date.. The inspection covered Choose an item. and was classified Choose an item.. ☒ An analysis of the firm's inspection history over the past 2 years showed that it has not been inspected. ☐ N/A - the manufacturing site does not require an inspection at this time given the risk of the combination product		
<u>Inspection Recommendation:</u> ☒ A pre-approval inspection <u>is required</u> because: The firm is responsible for major activities related to the manufacturing and/or development of the final combination involving the device constituent part; and, A recent medical device inspection of the firm <u>has not been performed</u>. ☐ An inspection <u>is not required</u> because Choose an item.		

5.3. Quality System Documentation Triage Checklist

Was the last inspection of the finished combination product manufacturing site, or other site, OAI for drug or device observations?	☐ Yes ☒ No ☐ UNK
Is the device constituent a PMA or class III device?	☐ Yes ☒ No ☐ UNK
Is the final combination product meant for emergency use?	☒ Yes ☐ No ☐ UNK
Is the combination product meant for a vulnerable population (infants, children, elderly patients, critically ill patients, or immunocompromised patients)?	☐ Yes ☒ No ☐ UNK
Does the manufacturing site have a significant and known history of multiple class I device recalls, repeat class II device recalls, a significant number of MDRs/AEs, or OAI inspection outcomes?	☐ Yes ☒ No ☐ UNK
Is the combination product meant for users with a condition in which an adverse event will occur if the product is not delivered correctly (example insulin products for specific diabetic patients)?	☒ Yes ☐ No ☐ UNK
Does the manufacturing process for the combination product device constituent part use unique, complicated, or not well understood methods of manufacturing?	☐ Yes ☒ No ☐ UNK
cGMP Risk:	
☐ Low or Moderate Risk of cGMP issues: If yes is not checked above, please fill out the checklist and deficiencies only. A review summary is optional.	
☒ High Risk of cGMP issues: If yes is checked anywhere above, consider filling out the checklist, the deficiencies, and the review summary. If a full review is not warranted due to other factors such as device constituent classification (class I and class II devices), a low or moderate overall risk of device constituent failure, or positive compliance history, please document your rationale below for not conducting a full ICCR review.	

Reviewer Comment

Since this Combination Product is intended for Emergency Use, non-compliance with the cGMP/QS systems represents a high risk and a desk review of the provided Quality Systems documentation is necessary.

Inspection Recommendation:

(b) (4) manufacturer of the device constituent parts of the combination product and hold the design history file for the device. This firm has not had a medical device inspection over the last 2 years. Since the device is intended to be used in emergency situations cGMP issues represent a significant risk and a medical device inspection is recommended.

Rechon Life Science AB is the manufacture of the final, finished device and is responsible for the assembly of the device subassemblies as well as the release and stability testing of the autoinjector. A review of the most recent inspection from (b) (4) indicated no inspectional coverage for Auto-Injector assembly or Auto-Injector testing capabilities. In addition, it does not appear that medical device coverage has ever been requested for this facility. Given the responsibilities of the facility, a review of the CAPA procedures and Design Control activities is recommended.

5.4. Filing Review Conclusion

FILING REVIEW CONCLUSION	
Acceptable for Filing: ☒ Yes ☐ No (Convert to a RTF Memo) ☐ N/A	
Facilities Inspection Recommendation:	
☒ (PAI) Pre-Approval Inspection ☐ Post-Approval Inspection ☐ Routine Surveillance	

No Inspection N/A

Site(s) needing inspection:

(b) (4)

Rechon Life Science AB (FEI: 3002806978)

Reviewer Comments

The submission is acceptable for filing. As discussed above, a 74-Day letter deficiency is recommended to request the missing test reports for all device verification testing.

Refuse to File Deficiencies: Yes No N/A

74-Day Letter Deficiencies: Yes No N/A

	Date Sent: 5/13/2020	Date/Sequence Received: 6/1/2020 Seq. 008
Information Request #1	You provided a summary of your device verification activities for the Pre-Filled Syringe and Auto-Injector. However, you have not provided full test protocols including a description of the test methods and data. This information is necessary for the review of the submission. Provide full test reports for the conducted verification activities of the device constituent parts of the combination product. Test reports should include a description of the test method, a description of the test article, and test results.	
Sponsor Response	Reviewed in Design Verification Section	

6. LABELING

6.1. General Labeling Review

The labeling, including the device constituent labeling, user guides, patient information, prescriber information and all other labeling materials provided for review were reviewed to meet the following general labeling guidelines as appropriate:

General Labeling Review Checklist	Adequate?		
	Yes	No	N/A
Indications for Use or Intended Use; including use environment(s); route(s) of administration for infusion, and treatment population.	X		
Drug name is visible on device constituent and packaging	X		
Device/Combination Product Name and labeling is consistent with the type of device constituent	X		
Prescriptive Statement/Symbol on device constituent	X		
Warnings	X		

Contraindications	X		
Instructions for Use	X		
Final Instructions for Use Validated through Human Factors	X (deferred to DMEPA)		
Electrical Safety Labeling/Symbols			X
EMC Labeling/Symbols			X
Software Version Labeling			X
MRI Labeling/Symbols			X
RF/Wireless Labeling/Symbols			X

Reviewer Comments

The provided labeling contains all necessary information. The provided human factors engineering report and study results to ensure the adequacy of the provided instructions are deferred to DMEPA.

6.2. Device Specific Labeling Review

Device Specific Labeling Review Checklist	Adequate?		
	Yes	No	N/A
Indications for Use or Intended Use; including use environment(s); route(s) of administration for infusion, and treatment population.	X		
Drug name is visible on device constituent and packaging	X		
Device/Combination Product Name and labeling is consistent with the type of device constituent	X		
Prescriptive Statement/Symbol on device constituent	X		
Warnings	X		
Contraindications	X		
Instructions for Use	X		

Reviewer Comments

The labeling contains all required elements (e.g., RX statement, indications for use, name of the drug, etc.) and the instructions for use are appropriate. The labeling states that it is a single dose product and that it should be discarded after use. The labeling is acceptable from a device perspective. Please note that a human factors evaluation and a detailed review of the step by step instructions for use of the devices is deferred to DMEPA.

6.3. Clinical Labeling Review

The following Clinical Labeling Review was completed by ☐ Insert Consultant Name ; The full memo is located in Appendix B. ☒ The Lead Reviewer Below is a summary of the review & recommendation:
The instructions for use are appropriate for the type of device. Please note that the human factors review is deferred to DMEPA. The IFUs for both the safety syringe and autoinjector were provided and no device specific clinical labeling concerns were identified.

6.4. Labeling Review Conclusion

LABELING REVIEW CONCLUSION		
Filing Deficiencies: ☐ Yes ☒ No ☐ N/A	Mid-Cycle Deficiencies: ☐ Yes ☒ No ☐ N/A	Final Deficiencies: ☐ Yes ☒ No ☐ N/A
<u>Reviewer Comments</u> The provided labeling is appropriate for the device type. A usability review of the instructions and device design is deferred to DMEPA.		
CDRH sent Labeling Deficiencies or Interactive Review Questions to the Sponsor: ☐ Yes ☒ No		

7. DESIGN CONTROL SUMMARY

7.1. Summary of Design Control Activities

Risk Analysis Attributes	Yes	No	N/A
Risk analysis conducted on the combination product	X		
Hazards adequately identified (e.g. FMEA, FTA, post-market data, etc.)	X		
Mitigations are adequate to reduce risk to health			
Version history demonstrates risk management throughout design / development activities			
Design Inputs/Outputs	Yes	No	N/A
Design requirements / specifications document present (essential performance requirements included)	X		
Design Verification / Validation Attributes	Yes	No	N/A
Validation of essential requirements covered by clinical and human factors testing	X		
To-be-marketed device was used in the pivotal clinical trial	X		
Bioequivalence Study utilized to-be-marketed device			
Verification methods relevant to specific use conditions as described in design documents and labeling	X		
Device reliability is acceptable to support the indications for use (i.e. emergency use combination product may require separate reliability study)	X		
Traceability demonstrated for specifications to performance data	X		

Reviewer Comments

The overall design control process is acceptable. Please see Section 8 for a review of the risk analysis that includes a general overview of the design control activities.

7.2. Design Inputs and Outputs

Essential Performance Requirements

<u>Design Inputs</u> (Essential Performance Requirement)	Design Outputs (Specification)
PFSS	
Deliverable volume	(b) (4)
Break loose force	
Glide force	
AI	
Dose accuracy	
Cap removal force	
Injection time	
Activation force	
Activation resistance force	
Needle extension	

Reviewer <u>Comments</u>
The provided EPRs are consistent with similar devices and are acceptable. Strict control of the EPRs will ensure functionality of the device.

7.3. Applicable Standards and Guidance Documents

Generally Applicable Standards and Guidance Documents:

Standard or Guidance	Conformance (Y/N/NA)
AAMI / ANSI / ISO 14971:2007/(R)2010 (Corrected 4 October 2007), medical devices - applications of risk management to medical devices	Y
Standard Practice for Performance Testing of Shipping Containers and Systems; ASTM D4169-09	Y
IEC 60601-1-2:2014	N/A
Guidance for Industry and FDA Staff: Current Good Manufacturing Practice Requirements for Combination Products (2017)	Y
Mobile Medical Applications Guidance for Industry and Food and Drug Administration Staff (2015)	N/A
Guidance for Industry and FDA Staff – Medical Devices with Sharps Injury Prevention Features (2005)	Y
Use of International Standard ISO 10993-1, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process"	Y
Applying Human Factors and Usability Engineering to Medical Devices	Y

Device Specific Standards and Guidance Documents

Standard or Guidance	Recognized (Y/N/NA)	Conformance (Y/N/NA)
ISO 10993-1:2018 <i>Biological Evaluation of Medical Devices – Part 1: Evaluation and Testing Within a Risk Management Process</i>	Y	Y
ISO 9626 <i>Stainless steel needle tubing for the manufacture of medical devices – Requirements and test methods</i>	Y	Y
USP <381> - Elastomeric Closure for Injections	N	Y
ISO 11040-4:2015 <i>Glass barrels for injectables and sterilized subassembled syringes ready for filling</i>	Y	Y

ISO 11608-1:2015 <i>Needle-based injection systems for medical use – Requirements and test methods – Part 1: Needle-based injection systems</i>	Y	Y
ISO 23908:2011 <i>Sharps injury protection – Requirements and Test Methods- Sharps Protection Features for single-use hypodermic needles, introducers for catheters and needles used for blood sampling.</i>	Y	Y

7.4. Design Control Review Conclusion

DESIGN CONTROL REVIEW CONCLUSION		
Filing Deficiencies: ☐ Yes ☒ No ☐ N/A	Mid-Cycle Deficiencies: ☐ Yes ☒ No ☐ N/A	Final Deficiencies: ☐ Yes ☒ No ☐ N/A
Reviewer Comments		
CDRH sent Design Control Deficiencies or Interactive Review Questions to the Sponsor: ☐ Yes ☒ No		

8. RISK ANALYSIS

8.1. Risk Management Plan

A risk summary was provided for both the Autoinjector and the Safety Syringe in 3.2.R – “Device – Product Risk Summary”.

The following provides an overview of the implemented risk management plan:

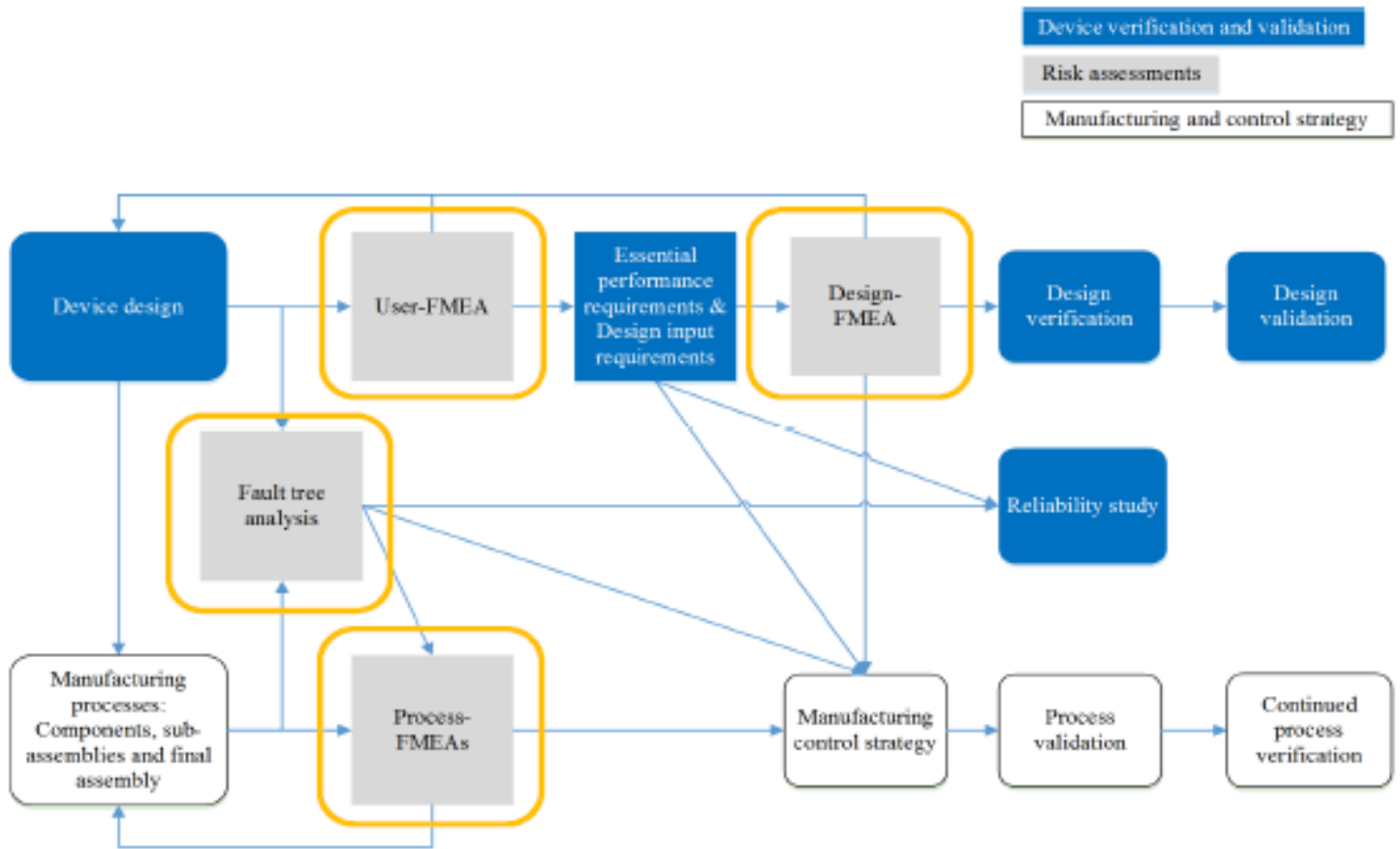


Figure 1 Risk based control overview

The risk management plan is divided into a User-FMEA, Design-FMEA, Process-FMEA, and a Fault Tree Analysis (FTA). Since this device is intended to be used by a lay user in an emergency situation, the Sponsor should demonstrate that the device is sufficiently reliable to ensure that the device is able to successfully deliver the intended dose. Feedback was previously provided to Zealand that the Agency expects a reliability of at least 99.999% that the device delivers the intended dose with a 95% confidence. In response, Zealand provided an FTA to support the reliability specification.

The following graph was provided indicating how the individual risk analysis/management activities are interconnected:

Note: Zealand Pharma has overall quality oversight for the product and for the risk management process

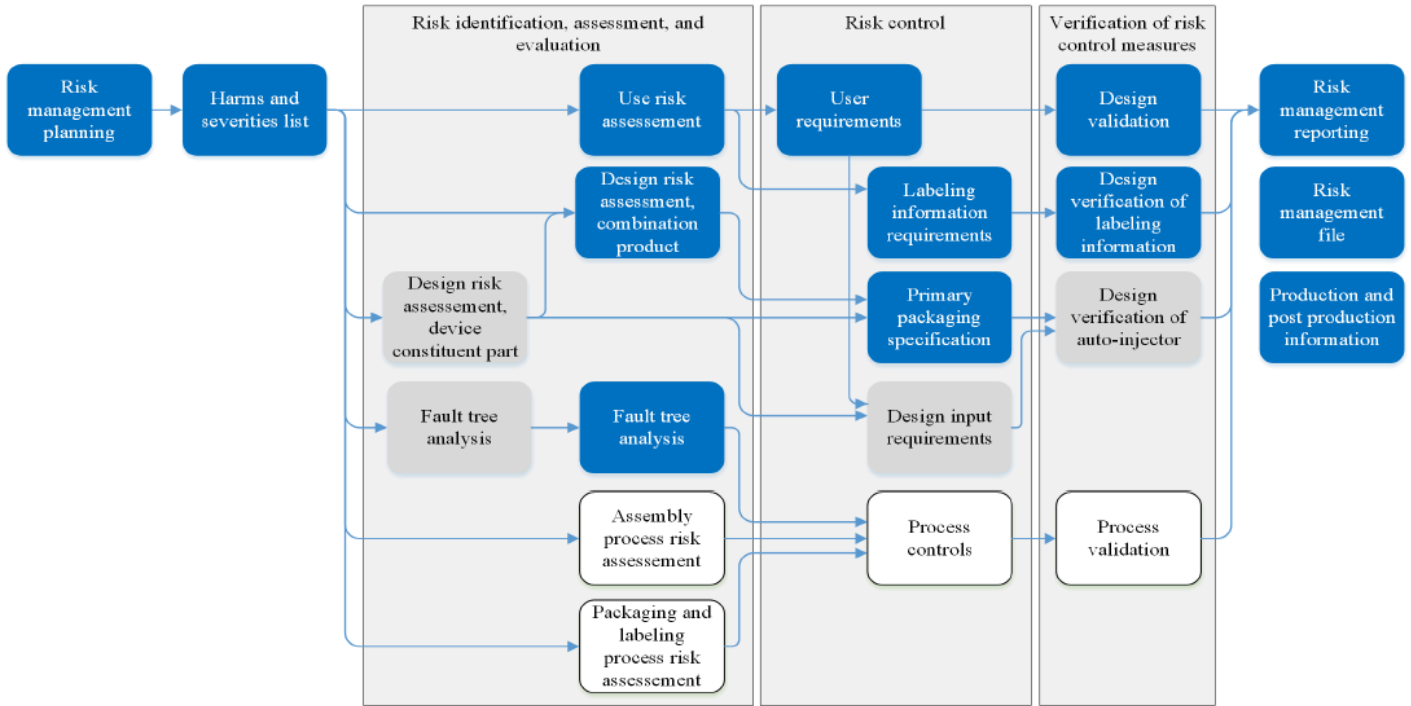


Figure 2 Overview of risk management processes

The following risk levels were defined by Zealand and are used in the risk assessments:

Table 2 Risk levels

Risk Level	Definition
High	High risk level. Unacceptable. Shall be reduced as far as possible. If risks in the high risk level cannot be further mitigated, a formal risk/ benefit analysis shall be performed to determine whether or not the medical benefit outweighs the residual risk.
Medium / Moderate	Medium risk level. Acceptable. Shall be reduced as far as possible.
Low	Low risk level. Acceptable. Shall be reduced as far as possible.
No Risk	No risk level. Acceptable. The risk cannot occur or has been eliminated.

Zealand separated probabilities of occurrence into two categories: probability of occurrence and probability of harm. The following definitions of probabilities were provided:

Table 3 Probabilities for design risk assessment

	The probability of a hazardous situation occurring (P1)	The probability of the hazardous situation leading to harm (P2)
Very high	Likely to occur frequently in the product population	Very likely to lead to harm to the end user

High	Likely to occur repeatedly in the product population	Likely to lead to harm to the end user
Moderate	Likely to occur sometime in the product population	May be likely to lead to harm to the end user
Low	Not likely to occur, but possible in the product population	Not likely, but possible, to lead to harm to the end user
Very low	Not likely to occur in the product population	Not likely to lead to harm to the end user

These probabilities were combined into the following table:

Table 4 Combined probabilities of harm for design risk assessment

P2	P1				
	Very low	Low	Moderate	High	Very high
Very high	L	L	M	H	VH
High	VL	L	L	M	H
Moderate	VL	VL	L	L	M
Low	VL	VL	VL	L	L
Very low	VL	VL	VL	VL	L

The following is a table defining the severities and the final acceptance criteria for the design risk assessment:

Table 5 Severities for design risk assessment

Definition	Severity
Results in death or life threatening injury	Very high
Results in permanent impairment or injury/impairment requiring medical intervention	High
Results in temporary injury or impairment not requiring medical intervention	Moderate
Results in inconvenience or temporary discomfort	Low
Results in dissatisfaction without any additional negative effect	Very low

Table 6 Acceptance criteria for design risk assessment

Probability of occurrence	Severity				
	Very low	Low	Moderate	High	Very high
Very high	Moderate	High	High	High	High
High	Low	Moderate	High	High	High
Moderate	Low	Moderate	Moderate	High	High
Low	Low	Low	Moderate	High	High
Very Low	Low	Low	Low	Moderate	Moderate

Reviewer Comments

The risk assessment was reviewed and is acceptable. The Sponsor identified reasonable design requirements and links them to risk control measures. For example, one identified risk is that the user drops the AI and cannot perform the injection while suffering severe hypoglycemia. The severity of the risk was classified as high and the probability of

occurrence as low. Risk control measures include design verification testing after vibration and drop pre-conditioning. This appears reasonable.

8.2. Hazard Analysis and Risk Summary Report

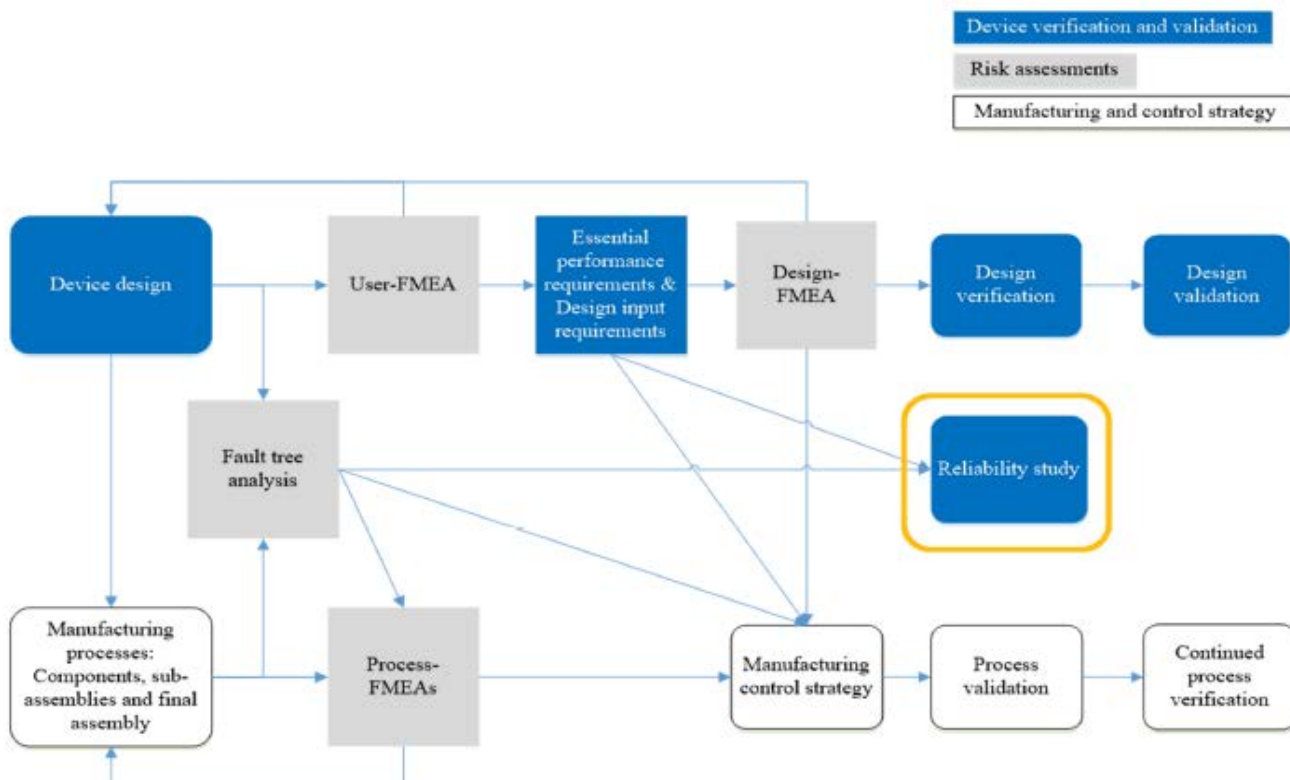
[Link to Infusion Pump SAC Reviewer Guide](#)

Both the AI and PFSS are intended to be used in an emergency situation by a lay user. Hence, there are additional device reliability considerations as the device is intended for a potentially life-saving application with no other easily accessible alternative.

8.2.1. Review of Device Reliability

The Sponsor provided information regarding the device reliability in 3.2.R Device – Reliability Performance Report – Auto-injector and in 3.2.R Device – Reliability Performance Report – Pre-filled Safety Syringe. There were several interactions with the Sponsor prior to the submission of this NDA. The Agency agreed that the reliability requirements for the PFSS will be specified as 99.99% reliable for a successful activation with 95% confidence and the AI be 99.999% reliable in terms of a successful activation with 95% confidence. Please note that a draft guidance was published in April of 2020 titled *Technical Considerations for Demonstrating Reliability of Emergency-Use Injectors Submitted under a BLA, NDA or ANDA: Guidance for Industry and Food and Drug Administration Staff*. This guidance specifically recommends a reliability of 99.999% which is based on risk analyses based on the FDA recognized ISO 14791. Although this guidance does not specifically apply to prefilled syringes, it is stated that recommendations in this guidance may be helpful in the assessment of syringes. However, since the Agency has interacted with this Sponsor extensively in the past and specifically recommended a 99.99% reliability specification for the PFSS, the chosen specification is acceptable.

Outline of design control process:



As recommended, the Sponsor conducted the reliability assessment by creating a fault-tree analysis (FTA) with the top level failure being “failure to fire” of the injector/syringe. The FTA is based on available manufacturing data and other estimates of component performance/dimensional analyses. The FTA was supplemented with device reliability testing of essential performance requirements after pre-conditioning. The following EPRs were identified for the devices with the associated control strategy:

EPRs Pre-filled Safety Syringe:

Table 2 EPR related process controls overview

Essential Performance Requirement	Specification	Reliability Test	Process Validation	Incoming Control *	In Process Control*	Release Testin	Stability Testing
Deliverable Volume	(b) (4)	√	√	-	√	√	√
Break Loose Force	(b) (4)	√	√	√	-	√	√
Glide Force	(b) (4)	√	√	√	-	√	√

* Controls are performed in the (b) (4) process as described in 3.2.P.3.4 Controls of Critical Steps and Intermediates and 3.2.P.5.1 Specification - Pre-filled Syringe.

EPRs Auto-injector:

Table 2 EPR related process controls overview

EPR	Specification	Reliability test	Process validation	Incoming control	In-process control	Release*	Stability testing
Dose accuracy	(b) (4)	√	√	-	√	√	√
Cap removal force	(b) (4)	√	√	-	√	√	√
Injection time	(b) (4)	√	√	-	√	√	√
Activation force	(b) (4)	√	√	CoA	√	√	√
Activation resistance force	(b) (4)	√	√	CoA	√	√	√
Needle extension	(b) (4)	√	√	CoA	√	√	√

* Test results generated during in-process controls will be used for batch release

Reliability requirements PFSS:

Table 3 k-factors for one and two sided tolerance intervals

	Essential Performance Requirements	Least Reliable Base Case
n=60	PC = 99.99% CL = 95%	PC = 99.99918 % CL = 95%
One sided		(b) (4)

Reliability requirements AI:

Table 3 Additional reliability requirement with CL=95% and PC=99.999%

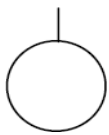
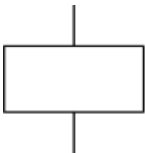
Reliability requirement	Definition	Requirement
Ability to fire	The definition of ability to fire is successful activation i.e. the plunger rod in the rear subassembly is released from its initial position in the rear subassembly and pushed forward by the power spring to cause the PFS to empty	Device fires

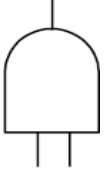
Table 4 k-factors for one and two sided tolerance intervals

	Essential Performance Requirements	Least Reliable Base Case
n=60	PC= 99.99 % C= 95%	PC= 99.99985 % C= 95%
One sided		(b) (4)
Two-sided		

Fault Tree Analysis:

The FTA was constructed using a set of four logic symbols: The basic event, event, OR gate, and AND gate. The following description of these symbols and associated computation instructions are provided below:

Symbol	Name	Description	Reliability correlation
	Basic event	The lowest level event for which probability of occurrence or reliability information is available	Input
	Event	Categories of the failure modes/Additional information	N/A

	OR Gate	The output event occurs if any of the input events occur	Output = Σ (Input)
Symbol	Name	Description	Reliability correlation
	AND Gate	The event occurs only if all input events occur	Output = Input ₁ *Input ₂ *...Input _n

The FTA of the AI is structured into failure modes related to a failure to activate the piston and unintentional activation (premature firing). The failure to activate piston branch is further divided into failures associated with the removal of the rigid needle shield (RNS), Needle Cover (NC) compression failure, and plunger rod deployment failure.

The auto-injector operates by applying the required activation force onto the needle cover. (b) (4)
 (b) (4)
 (b) (4). Once fully activated, (b) (4)
 (b) (4) leading to the injection of the medication. Hence, it appears reasonable to divide the failure modes related to the mechanical interactions inside the device into failures related to the needle cover and the rear-subassembly (b) (4)
 (b) (4).

For this review, three failures were traced down to the base events and evaluated for adequacy. Two of these were chosen from the AI FTA and one from the PFSS FTA. The following is a review of these failure modes:

Auto-injector Failure Mode 1.2.1.4 (b) (4)

The following is the trace of this failure mode in the FTA:

3 Pages have been Withheld in Full as B4(CCI/TS)
 Immediately Following this Page

Overall, the failure mode was adequately traced back to the component. A rationale was provided that the yield stress ^(b)₍₄₎ ^(b)₍₄₎ is large compared to the activation force of the injector. Activation force testing was provided to support this rationale. The failure rate was estimated for the component and incorporated in the overall FTA calculation. This is acceptable.

PFSS Failure Mode 1.3.2.2 Particles in barrel

The failure was traced the following way:



The following higher level failure modes were identified for *1.3.2.2 Particles in barrel: 1.3.1 Barrel/tip clogged, 1.3 No drug delivery through needle or barrel, 1. Plunger not able to initiate or complete movement and PFSS unable to deliver full dose*. The place of this failure mode in the fault tree is reasonable. A particle in the barrel could lead to clogging of the syringe that could lead to incomplete plunger movement. The overall estimated failure rate for *Particles in barrel* is (b) (4) ppm. This was supported by the needle inner diameter specification, the customer quality specification from the component supplier (b) (4) and the in-process visual inspection control. Zealand stated that the glass particle specification of >(b) (4) μm has an AQL of (b) (4) particles of that size are expected to clog the needle due to dimensional constraints. This AQL level leads to a worst case failure rate of (b) (4) ppm. To further reduce the theoretical rate of failure, Zealand conducted special training of personal by inspecting 2 lots of 500 PFS three times where a critical fault must be found. Based on this training, Zealand adjusted the (b) (4) ppm failure rate by the estimated success rate to detect a failure during in-process inspections (b) (4) to (b) (4) ppm. This is acceptable. Zealand identified a need to improve in-process controls based on the FTA and reduced the theoretical failure rate.

FTA conclusion

The FTAs for the AI and PFSS appear to be adequately constructed and failure modes are traced back to the component level. In-process controls were implemented to reduce failure rates of certain failure modes. The overall stated reliability based on the two level goal of each FTA is:

AI = (b) (4) % reliable
PFSS = (b) (4) % reliable

This is in line with the recommendations communicated to the Sponsor and is adequate.

Performance testing to support the calculated device reliability

Zealand choose variable testing methods to support the reliability of the device. The sample sizes of each test were justified based on the tolerance interval statistical methodology and was guided by ISO 19269-6 *Determination of Statistical Tolerance Intervals*. Since this standard only provides tables of k values for associated reliabilities up to 99%, Zealand utilized the R function K.factor to calculate k values with associated higher reliabilities. In addition, calculations were done in excel to simplify the process. The results from the R function were compared to the values from ISO 19269-6 and were found to exactly align. Differences between the excel calculations and the R function calculations were plotted and found to be negligible:

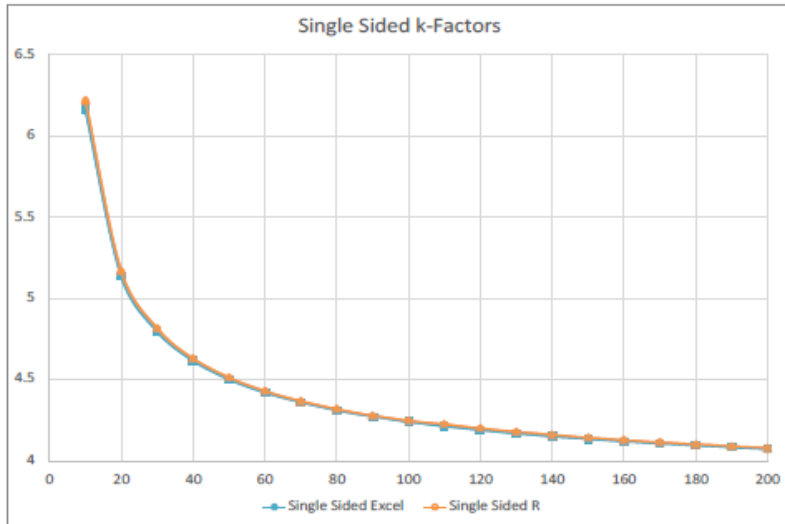


Figure 1 Single Sided k-Factors

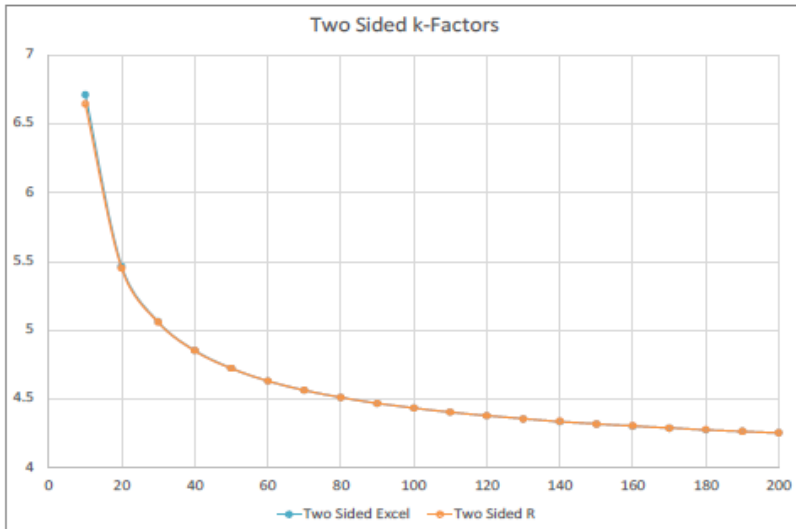
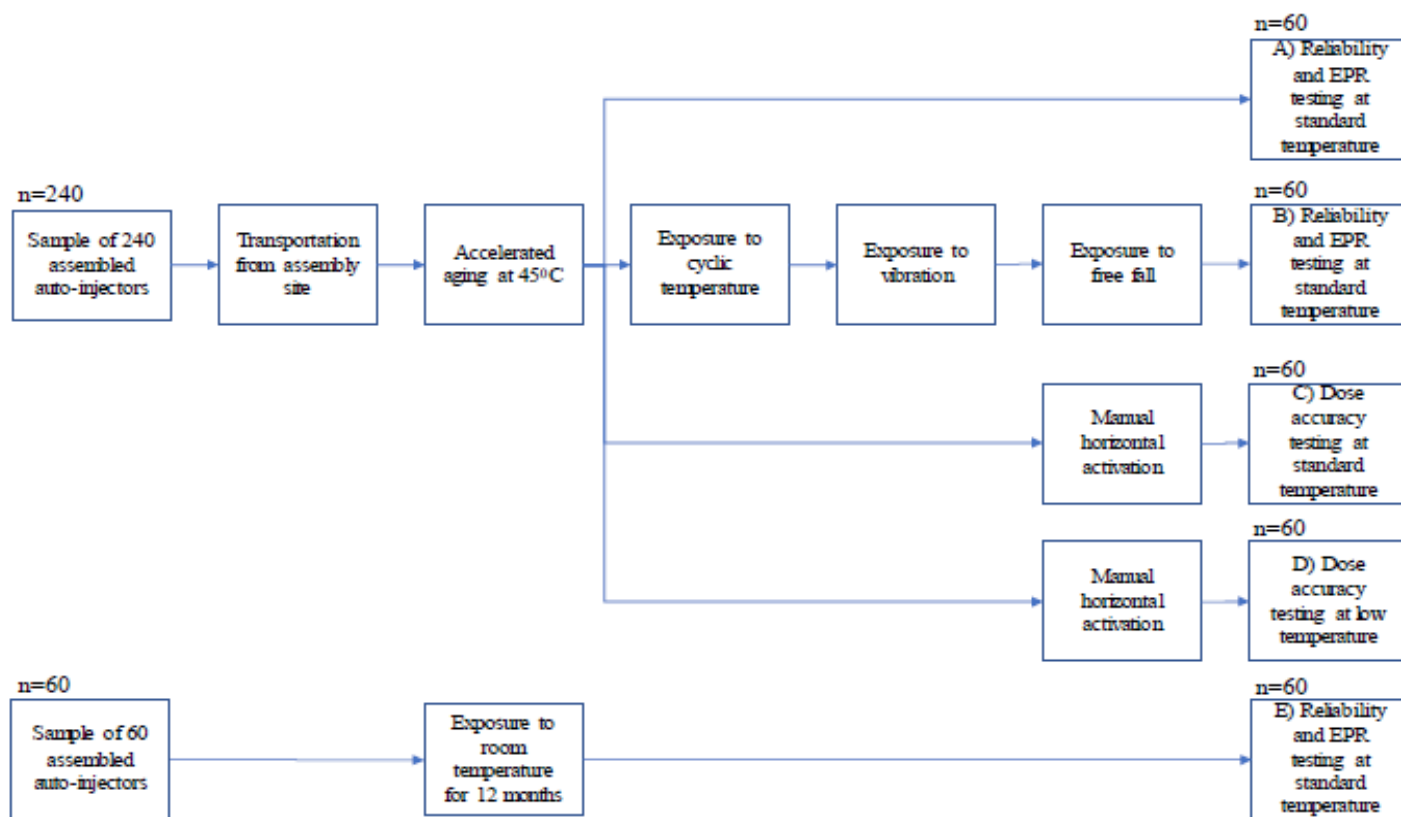


Figure 2 Two-sided k-Factors

The following test battery was conducted on the AI to support the reliability specification:



The following results were provided after preconditioning based on the 5 different scenarios listed in the flow chart above:

Auto-injector:

Table 6 Reliability performance test results (Exposure A in [Figure 2](#))

Test	k-factor	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL
Dose accuracy					(b) (4)	PASS
Cap removal force						PASS
Injection time						PASS
Activation force						PASS
Activation resistance force						PASS
Needle extension						PASS

Table 7 Reliability performance test results (Exposure B in [Figure 2](#))

Test	k-factor	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL

Dose accuracy		(b) (4)	PASS
Cap removal force			PASS
Injection time			PASS
Activation force			PASS
Activation resistance force			PASS
Needle extension			PASS

Table 8 Reliability performance test results (Exposure C in [Figure 2](#))

Test	k-factor	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL
Dose accuracy						(b) (4) PASS

Table 9 Reliability performance test results (Exposure D in [Figure 2](#))

Test	k-factor	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL
Dose accuracy						(b) (4) PASS

The results for the reliability success criteria defined in [3.3](#) are summarized in [Table 10](#).

Table 10 Reliability performance results

Test	Target	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL
Dimension AB - Exposure A (see Appendix 2 Sample Size Justification)						(b) (4) PASS
Dimension AB - Exposure B (see Appendix 2 Sample Size Justification)						PASS
# of auto-injectors that fired						PASS

Table 11 EPR performance after 12 months room temperature storage (Exposure E in [Figure 2](#))

Test	k-factor	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL
Dose accuracy						(b) (4) PASS
Cap removal force						PASS
Injection time						PASS
Activation force						PASS

Activation resistance force	(b) (4)	PASS
Needle extension	(b) (4)	PASS

Table 12 Reliability performance after 12 months room temperature storage (Exposure E in [Figure 2](#))

Test	Target	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/ FAIL
Dimension AB - Exposure E (see Appendix 2 Sample Size Justification)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS
# of auto-injectors that fired	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS

Pre-filled Safety Syringe

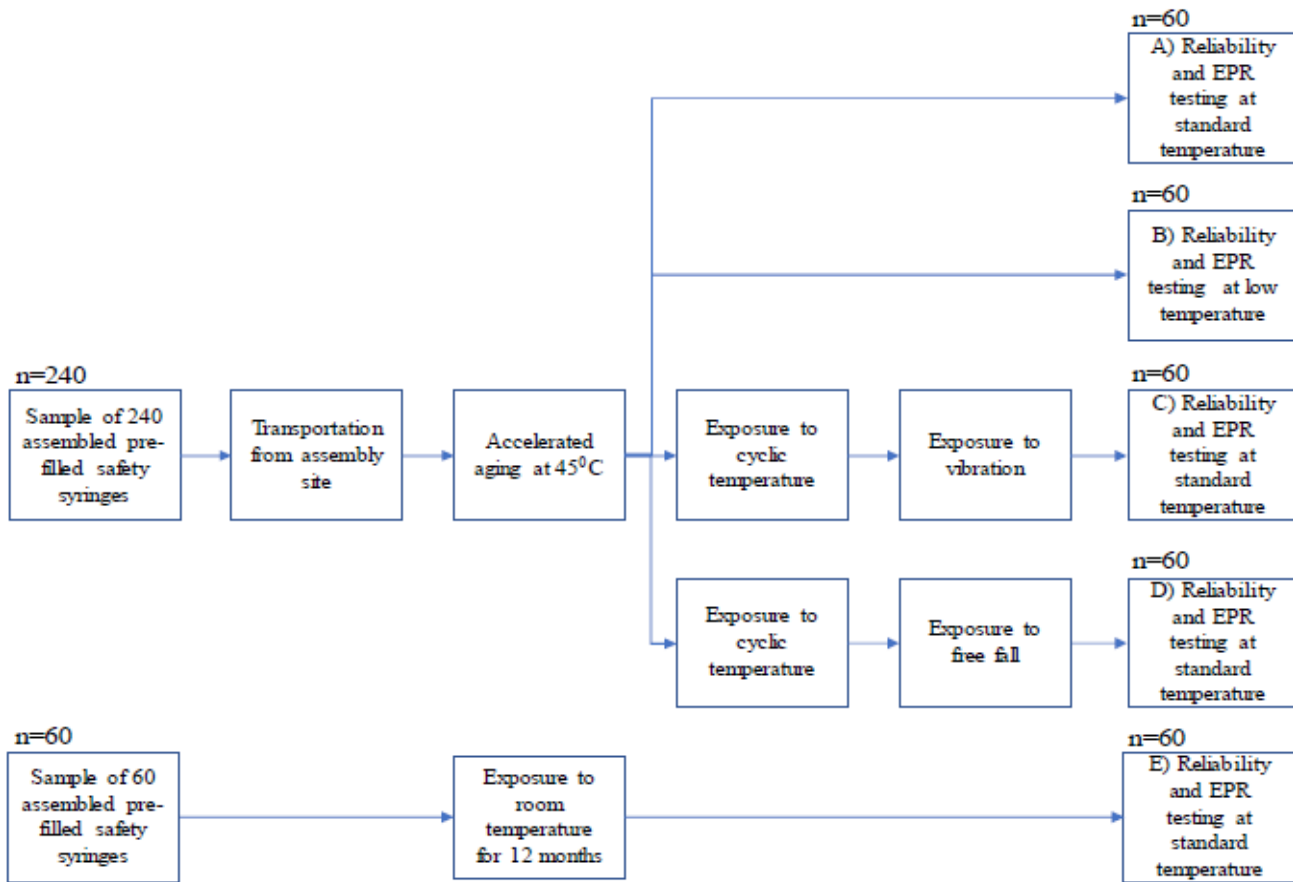


Table 5 Reliability performance test results (Exposure A in [Figure 2](#))

Test	k-factor	Mean	SD	Tolerance Interval limit	Acceptance Criteria	PASS/ FAIL
Break loose force	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS

Glide Force	(b) (4)	PASS
Deliverable volume	(b) (4)	PASS

Table 6 Reliability performance test results (Exposure B in [Figure 2](#))

Test	k-factor	Mean	SD	Tolerance Interval limit	Acceptance Criteria	PASS/ FAIL
Break loose force	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS
Glide Force	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS
Deliverable volume	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS

Table 7 Reliability performance test results (Exposure C in [Figure 2](#))

Test	k-factor	Mean	SD	Tolerance Interval limit	Acceptance Criteria	PASS/ FAIL
Break loose force	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS
Glide Force	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS
Deliverable volume	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS

Table 8 Reliability performance test results (Exposure D in [Figure 2](#))

Test	k-factor	Mean	SD	Tolerance Interval limit	Acceptance Criteria	PASS/ FAIL
Break loose force	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS
Glide Force	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS
Deliverable volume	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS

Table 9 EPR performance after 12 months room temperature storage (Exposure E in [Figure 2](#))

Test	k-factor	Mean	SD	Tolerance Interval limit	Acceptance Criteria	PASS/ FAIL
Break loose force	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS
Glide Force	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS
Deliverable volume	(b) (4)	(b) (4)	(b) (4)	(b) (4)	(b) (4)	PASS

In addition, injection through clothing was conducted with n=60 samples and no failures were reported.

Conclusion Performance Testing to Support Reliability

The sample size justification and k value calculations appear to be reasonable. Devices were preconditioned by aging, temperature cycling, handling, and dropping. In addition, misuse conditions were simulated by injection through clothing. Zealand has not reported any failures during the performance testing and has adequately supported the reliability specifications for the PFSS and AI with the battery of tests.

Reviewer Comments

The provided reliability reports for the PFSS and AI were reviewed and found to be acceptable. The Sponsor demonstrated through the FTA that the PFSS is delivered with a reliability of greater than 99.99% with respect to the full dose delivered and the AI is delivered with a reliability of greater than 99.999% with respect to “failure to fire”. The conducted reliability performance testing was reviewed and found adequate (see above). Hence, the risk analysis and demonstration of device reliability is acceptable.

8.3. Risk Analysis Review Conclusion

RISK ANALYSIS REVIEW CONCLUSION		
Filing Deficiencies: ☐ Yes ☒ No ☐ N/A	Mid-Cycle Deficiencies: ☐ Yes ☒ No ☐ N/A	Final Deficiencies: ☐ Yes ☒ No ☐ N/A
<u>Reviewer Comments</u> Please see review conclusions above. The risk analysis and device reliability were found to be acceptable.		
CDRH sent Risk Analysis Deficiencies or Interactive Review Questions to the Sponsor: ☐ Yes ☒ No		

9. DESIGN VERIFICATION REVIEW

9.1. Performance/Engineering Verification

9.1.1. Essential Performance Requirement Evaluation

Essential Performance Requirement (Design Input)	Specification (Design Output)	Verification Method <u>Acceptable</u> (Y/N)	<u>Validation</u> (Y/N)	Aging / Stability (Y/N)	Shipping/ Transportation (Y/N)
Pre-filled Safety Syringe					
Deliverable volume	(b) (4)	Yes. Gravimetric measurement (Density (b) (4) g/mL).	Y. Clinical Study	Y	Y
Break loose force		Yes. Force Measurement at standard temperature.	Y. Clinical study/HF Study (see 9.1.3 for details)	Y	Y
Glide force		Yes. Force Measurement at standard temperature.	Y. Clinical study/HF Study (see 9.1.3 for details)	Y	Y
Auto-injector					
Dose accuracy	(b) (4)	Yes. Gravimetric measurement (Density (b) (4) g/mL).	Y. Clinical Study	Y	Y
Cap removal force		Yes. Force Measurement at standard temperature.	N. Clinical study/HF Study. However, actual cap removal forces are much lower than the specification. Hence, it is unclear how the high cap removal	Y	Y

			force is validated. See IR#2 below.		
Injection time	(b) (4)	Yes. Frame counting during a dose accuracy testing using video equipment.	Y. Clinical study/HF Study	Y	Y
Activation force		Yes. Force Measurement at standard temperature.	Y. Clinical study/HF Study	Y	Y
Activation resistance force		Yes. Force Measurement at standard temperature.	Y. Clinical study/HF Study	Y	Y
Needle extension		Yes. Testing in the fully depressed form during activation force testing.	Y. Clinical Study (see 9.1.3 for details)	Y	Y

Reviewer Comment

Shipping/Transportation:

Shipping was conducted per ASTM D4169-16 DC 13 assurance level I. This is acceptable. The following summary results were provided after shipping:

PFSS:

Table 7 Results after transportation

Verification	Method	Sample size	Acceptance criteria	Result
Visual inspection	Visual inspection	60	No dislocated parts, no cracks in the components of the device. Labels must be fully attached and all print must be legible. Cartons must be whole and undamaged.	Pass
Deliverable volume	Instrumental weighing	60	(b) (4) with 99.99% probability content with 95% confidence	Pass (b) (4)

Verification	Method	Sample size	Acceptance criteria	Result
Break loose force	Instrumental force	60	≤ (b) (4) N with 99.99% probability content	Pass

	measurement		with 95% confidence	(b) (4)
Glide force	Instrumental force measurement	60	$\leq$ (b) (4) N with 99.99% probability content with 95% confidence	Pass (b) (4)

AI:

Table 12 Summary of design verification after simulated transport of final assembled, packaged and labelled auto-injectors

Verification	Method	Sample size	Acceptance criteria	Result (Pass/Fail)
Visual inspection	Visual inspection	60	No dislocated parts, no cracks in the body and /or component of the device. Labels must be fully attached and all print must be legible. Cartons must be whole and undamaged.	Pass
Dose accuracy	Instrumental weighing	60	(b) (4) with 99.99% probability content with 95% confidence	Pass (b) (4)

Verification	Method	Sample size	Acceptance criteria	Result (Pass/Fail)
Cap removal force	Instrumental force measurement	60	$\leq$ (b) (4) N with 99.99% probability content with 95% confidence	Pass (b) (4)
Injection time	Video frame counting	60	$\leq$ (b) (4) seconds with 99.99% probability content with 95% confidence	Pass (b) (4)

Activation force	Instrumental force measurement	60	$\geq \frac{(b)(4)}{(4)}$ N with 99.99% probability content with 95% confidence	Pass (b)(4)
Activation resistance force	Instrumental force measurement	60	$\geq \frac{(b)(4)}{(4)}$ N with 99.99% probability content with 95% confidence	Pass (b)(4)
Needle extension	Vision control	60	$\geq \frac{(b)(4)}{(4)}$ mm and $\leq \frac{(b)(4)}{(4)}$ mm with 99.99% probability content with 95% confidence	Pass (b)(4)

Accelerated Aging:

Devices were aged for 52 days at 45°C and subsequently stored for 6 months at 25°C. While these storage conditions substantiate a 3 year storage period at 5°C, it is unclear how they substantiate the worst case storage conditions of 2 years at 5°C followed by 1 year at 25°C. Per the labeling, the device may be stored for up to 3 years at 5°C (b)(4). The Sponsor will be issued an IR to justify the accelerated aging conditions for the device constituents.

Hence, an IR is recommended. Please see IR#3 below for detailed.

9.1.2. Verification of Design Inputs Evaluation

<u>Design Input</u>	<u>Design Output</u>	<u>Verification Method</u>	<u>Results/Deviations</u>	<u>Adequately Verified (Y/N)</u>	<u>Validated through Clinical, Human Factors or Other</u>	<u>Adequately Validated (Y/N)</u>
Pre-filled Safety Syringe						

(b) (4)						
Protective case	The protective case is present.	Review.	Pass	Y	HF Study	Deferred to DMEPA
Protective case opening force	The protective case opening force enables the user to open the protective case, while preventing unintended opening. NLT ^(b) ₍₄₎ N, NMT ^(b) ₍₄₎ N	Instrumental force measurement.	Pass	Y	HF study	Deferred to DMEPA
Rigid needle shield removal force	The rigid needle shield removal force enables the user to remove the rigid needle shield, while preventing unintended removal of the rigid needle shield. NLT ^(b) ₍₄₎ N, NMT ^(b) ₍₄₎ N	Instrumental force measurement.	Pass	Y	HF study	Deferred to DMEPA
Relevant requirements of ISO 11040-8:2016	The PFSS complies with the relevant requirements of ISO 11040-8	Review of ISO 11040-8 checklist.	Pass	Y	N/A	N/A
(b) (4)						
Needle safety distance (no contact to 6 mm radius sphere)	A sphere of radius 6 mm (simulating a finger) cannot unintentionally access the needle tip.	Review of supplier documentation.	Pass	Y	N/A	N/A
Indication of full dose	The position of the plunger and the plunger rod indicates that the full dose has been injected	Review.	Pass	Y	Design verification	Yes

Inspection of the drug	The design allows the user to inspect the drug.	Review.	Pass	Y	Design verification	Yes
Protective case material	The material of the protective case is as specified.	Review.	Pass	Y	N/A	N/A
Size for fitting in a sharps disposal container	The used PFSS fits in a sharps disposal container to prevent unintended access to the needle tip.	Review.	Pass	Y	N/A	N/A
Exposed needle length	The exposed needle length is within specifications.	Microscope measurement.	Pass	Y	Clinical study	Yes
Needle gauge	The needle gauge is according to specifications.	Review of supplier documentation.	Pass	Y	Clinical Study	Yes
Protective case color	The color of the protective case is as specified.	Review.	Pass	Y	Design verification	Yes
Protective case dimensions	The dimensions of the protective case are below the specified threshold.	Review of supplier documentation.	Pass	Y	N/A	N/A
Weight	The weight of the PFSS in the protective case is below the specified threshold.	Instrumental weighing.	Pass	Y	N/A	N/A
Protective case sharp edges	The protective case does not have sharp edges that may injure the user.	Review of supplier documentation.	Pass	Y	N/A	N/A
PFSS sharp edges	The PFSS except for the needle tip does not have sharp edges that may injure the user.	Review.	Pass	Y	N/A	N/A
Protective case performance	The protective case protects the PFSS when being exposed to compression forces.	Instrumental force measurement.	Pass	Y	Design verification	Yes
Carton	The sales carton can contain the protective case and the labelling components.	Review.	Pass	Y	N/A	N/A

Auto-injector

Plunger rod force (at start and finish of injection)	Start: NLT ^(b) ₍₄₎ N, NMT ^(b) ₍₄₎ N Low Load: NLT ^(b) ₍₄₎ N, NMT ^(b) ₍₄₎ N N	Instrumental force measurement. The test is performed on qualified and calibrated equipment to verify that force profile of the AI rear sub-assembly is within specifications. ^(b) ₍₄₎	Pass	Yes	Clinical Study, HF	Yes
Cap removal minimum force ₁	NLT ^(b) ₍₄₎ N	Instrumental force measurement. Verifies the threshold for unintended removal of the cap. This is acquired during the EPR testing “cap removal force”.	Pass	Yes	Clinical Study, HF	Yes
Needle extension at activation	NLT ^(b) ₍₄₎	Vision control. The test is performed on qualified and calibrated vision control equipment with custom fixture to grip the auto-injector to verify the needle extension in mm when the drug is starting to be expelled.	Pass	Yes	Clinical Study, HF	Yes
Color specification of components	Color is as specified	Review. Verifies that the colors of the AI components are as specified. Pantone color codes are utilized to	Pass	Yes	HF	Yes

		qualify the approved samples.				
Needle guard deflection	NMT (b) (4) at (b) (4) N	Instrumental displacement measurement. The test is performed on qualified and calibrated equipment to detect the deflection of locked needle guard at (b) (4) N load and after gaps are eliminated as ≤ (b) (4) The custom fixture is set to hold the used AI with the needle guard locked in place. With a load of (b) (4) N, the axial movement is recorded.	Pass	Yes. Reviewer's note: Needle guard/cover deflection was analyzed as a variable test. However, when analyzing this attribute using constant force while measuring the needle guard displacement, it should be treated as an attribute test as the needle is either exposed or not. While this analysis was done as a variable test, the cumulative number of tested devices (240 during stability, 60 after transportation simulation, and 60 at standard conditions) support a passing result with 95% confidence and 99% reliability	HF	Yes

				when analyzed as an attribute test.		
Cap removal force (without syringe)	NMT ^(b) ₍₄₎ N, NLT ^(b) ₍₄₎ N	Instrumental force measurement. The test setting is similar to the EPR testing “cap removal force”. Verifies the AI components’ contribution to the cap removal force.	Pass	Yes	Clinical Study, HF	Yes
Front cap cover separation force	NLT ^(b) ₍₄₎ N	Instrumental force measurement. The test is performed on qualified and calibrated equipment in the application of custom fixture to grab front cap subassembly while as the force is applied to disassemble front cap and front cap cover. This is to verify the threshold for unintended disassembly of the cap.	Pass	Yes	Clinical Study, HF	Yes
Rear body separation force	NLT ^(b) ₍₄₎ N	Instrumental force measurement. The test is performed on qualified and calibrated equipment in the application of fixture for the separation of rear body from the AI. This is to verify the threshold for unintended disassembly of the AI.	Pass	Yes	Clinical Study, HF	Yes
Interface towards PFS	Tolerance analysis.	Verifies that the internal AI design is suitable for the PFS. This is defined	Pass	Yes	Design Verification	Yes

		as: the device shall be able to contain and operate a pre-filled syringe with specifications according to primary package specification. No collisions according to tolerance analysis (> ^(b) ₍₄₎ mm).				
Needle safety distance (no contact to 6 mm radius sphere)	Tolerance analysis.	Tolerance analysis. Verifies that a sphere of radius 6 mm (simulating a finger) cannot unintendedly access the needle tip. The needle shall not contact a sphere of radius 6 mm according to tolerance analysis in line with ISO 23908 Annex B. No contact, > ^(b) ₍₄₎ mm.	Pass	Yes	Design Verification	Yes
No contact between needle and needle guard	Tolerance analysis.	Tolerance analysis. Verifies that the needle guard does not interfere with the function of the needle. No contact between needle and needle guard (cover) (> ^(b) ₍₄₎ mm) according to tolerance analysis.	Pass	Yes	Design Verification	Yes
Needle visibility	Visual Inspection	Trained test engineer to verify that the needle is not visible to the user when viewing the AI perpendicular. Evaluated jointly by	Pass	Yes	Clinical Study, HF	Yes

		(b) (4) and ZP project team.				
Feedback completed injection	Tactile and audible feedback	Attribute test. Trained test engineer to verify that the AI gives audible feedback (click) at start and end of injection.	Pass	Yes	Clinical Study, HF	Yes
Drug visibility	Visual Inspection	Trained test engineer to verify that the medicine window allows the user to inspect the drug. Evaluated jointly by (b) (4) and ZP project team.	Pass	Yes	Clinical Study, HF	Yes
Ready for injection	Review	Trained test engineer to verify that the AI can only activate when the cap has been removed by the user. Evaluated jointly by (b) (4) and ZP project team.	Pass	Yes	Clinical Study, HF	Yes
Indication of used AI	Visual Inspection	Trained test engineer to verify that the medicine window is filled with the red plunger rod after injection to indicate that the AI has been used. Evaluated jointly by (b) (4) and ZP project team.	Pass	Yes	Clinical Study, HF	Yes
Handling steps	Review	Trained test engineer to verify that the AI can only activate when the cap has been removed by the user and the needle guard is pressed fully against the injection site. Evaluated	Pass	Yes	Clinical Study, HF	Yes

		jointly by (b) (4) and ZP project team.				
Sharp edges	Visual Inspection	Trained test engineer to verify that the AI does not have sharp edges that may injure the user. Evaluated jointly by (b) (4) and ZP project team.	Pass	Yes	Clinical Study, HF	Yes
Needle override force after free fall of used AI	NLT (b) (4)N, while Needle Cover displacement NMT (b) (4)	Instrumental force measurement. Verifies that the needle protection mechanism works after free fall to protect against unintended exposure to the needle tip.	Pass	Yes	Design Verification	Yes

Reviewer Comment

Test methods are reviewed below.

9.1.3. *Evaluation of Test Methods*

Title:	PFSS: Deliverable Volume
Scope/Objective & Acceptance Criteria:	$\geq$ (b) (4)
<u>Methods</u>	<i>The deliverable volume from the PFSS is measured according to the current ISO-11040-8 standard and is performed based on compendial test for extractable volume by gravimetric method based on USP <697> and Ph. Eur. 2.9.17.</i> <i>The only exception to the compendial test is an increased sample size at release testing (as in accordance with specific reliability criteria) compared to sample size required in the compendial procedure (i.e. n=5).</i>

	<i>In summary, the test is performed on a qualified and calibrated test equipment. The syringe is emptied into a collection vessel using a plunger rod. The collection vessel is weighed before and after the injection and the difference in weight is converted to volume using the density of the drug. Density of the drug used to convert mass to volume is (b) (4).</i> Sample size: 60 samples (see reliability section above for more details regarding sample size)																									
Results:	Table 2 Deliverable volume results <table border="1"> <thead> <tr> <th>Pre-conditioning</th> <th>Acceptance criteria</th> <th>Sample size</th> <th>Average mL</th> <th>Minimum mL</th> <th>Maximum mL</th> <th>Stand-and deviation</th> <th>Result</th> </tr> </thead> <tbody> <tr> <td>Accelerated aging followed by preconditioning to standard temperature</td> <td rowspan="4">(b) (4) with 99.99% probability content with 95% confidence</td> <td>60</td> <td rowspan="4"></td> <td rowspan="4"></td> <td rowspan="4"></td> <td>(b) (4)</td> <td>Pass</td> </tr> <tr> <td>Accelerated aging followed by preconditioning to low temperature</td> <td>60</td> <td>Pass</td> </tr> <tr> <td>Accelerated aging followed by vibration and preconditioning to standard temperature</td> <td>60</td> <td>Pass</td> </tr> <tr> <td>Accelerated aging followed by free fall and preconditioning to standard temperature</td> <td>60</td> <td>Pass</td> </tr> </tbody> </table>	Pre-conditioning	Acceptance criteria	Sample size	Average mL	Minimum mL	Maximum mL	Stand-and deviation	Result	Accelerated aging followed by preconditioning to standard temperature	(b) (4) with 99.99% probability content with 95% confidence	60				(b) (4)	Pass	Accelerated aging followed by preconditioning to low temperature	60	Pass	Accelerated aging followed by vibration and preconditioning to standard temperature	60	Pass	Accelerated aging followed by free fall and preconditioning to standard temperature	60	Pass
Pre-conditioning	Acceptance criteria	Sample size	Average mL	Minimum mL	Maximum mL	Stand-and deviation	Result																			
Accelerated aging followed by preconditioning to standard temperature	(b) (4) with 99.99% probability content with 95% confidence	60				(b) (4)	Pass																			
Accelerated aging followed by preconditioning to low temperature		60				Pass																				
Accelerated aging followed by vibration and preconditioning to standard temperature		60				Pass																				
Accelerated aging followed by free fall and preconditioning to standard temperature		60				Pass																				
Conclusions/ Reviewer Comments:	Dose accuracy of the syringe is determined gravimetrically. The results support the chosen reliability specification and are acceptable. Dose accuracy specification is validated through clinical studies.																									
Acceptable:	☒ Yes ☐ No																									
Title:	PFSS: Breakloose and Glide force																									
Scope/Objective & Acceptance Criteria:	Breakloose: $\leq$ (b) (4) N Glide Force: $\leq$ (b) (4) N																									
Methods	<i>The break loose and glide force in Newtons is measured according to the current ISO 11040-8 standard, Section 6.2 (extrusion force).</i>																									

The break loose force is measured via a force testing machine with a calibrated load cell. The break loose force is defined as the peak force needed to overcome the static friction between the syringe plunger (stopper) and the glass barrel when the plunger is displaced at a fixed velocity of (b) (4).

The glide (expulsion) force is measured via a force testing machine with a calibrated load cell. The glide force is defined as the peak force after the initial displacement of the plunger at a fixed velocity of (b) (4). The glide force is interpreted according to the current ISO 11040-4 standard.

Sample size: 60 samples (see reliability section above for more details regarding sample size)

Results:

Table 3 Break loose force results

Pre-conditioning	Acceptance criteria	Sample size	Average N	Minimum N	Maximum N	Standard deviation	Result
Accelerated aging followed by preconditioning to standard temperature	≤ (b) (4)N with 99.99% probability content with 95% confidence	60	(b) (4)				Pass
Accelerated aging followed by preconditioning to low temperature		60					Pass
Accelerated aging followed by vibration and preconditioning to standard temperature		60					Pass
Accelerated aging followed by free fall and preconditioning to standard temperature		60					Pass

Table 4 Glide force results

Pre-conditioning	Acceptance criteria	Sample size	Average N	Minimum N	Maximum N	Standard deviation	Result
Accelerated aging followed by preconditioning to standard temperature	≤ (b) (4)N with 99.99%	60	(b) (4)				Pass

	Accelerated aging followed by preconditioning to low temperature	probability content with 95% confidence	60	(b) (4)	Pass	
	Accelerated aging followed by vibration and preconditioning to standard temperature		60		Pass	
	Accelerated aging followed by free fall and preconditioning to standard temperature		60		Pass	
Conclusions/ Reviewer Comments:	Breakloose and glide force testing was conducted using a load cell. Peak force and sustained forces were measured at a rate of (b) (4). The specification of NMT (b) (4) N were validated through the clinical trial and human factors study. The BL/GF data shows that the actual forces are much lower than the specification (b) (4) N). Hence, devices used in the validation studies likely had a much lower activation force. However, pre-filled syringes with similar BL/GF have been previously approved (e.g., ANDA 205935 or BLA 125559). In addition, note that the adequacy of the Human Factors study is deferred to DMEPA.					
Acceptable:	☒ Yes ☐ No					

Title:	AI: Dose Accuracy
Scope/Objective & Acceptance Criteria:	The dose accuracy test conducted for design verification was performed by (b) (4) according to their protocol (This test verifies that the dose accuracy is within specification. The test was performed according to ISO 11608-1:2014 section 10.1 and 11.3. The test was performed after preconditioning according to ISO 11608-1:2014 section 10.2 cool, standard, and warm atmosphere, section 10.5 free fall, section 10.6 dry heat and cold storage, and section 10.9 vibration. Acceptance Criterion: $\geq$ (b) (4)
Methods	The test is performed on a qualified and calibrated test equipment with custom fixture to grip the auto-injector vertically and activates the auto-injector to deliver the dose and that performs instrumental weighing of the expelled drug product. Doses were weighed and converted to volume using the density of the drug. In order to evaluate the dose accuracy, the one-sided statistical tolerance limit was calculated using the mean ($\bar{x}$) minus the standard deviation (s) multiplied by the tolerance limit factor (k) as described in ISO 11608-1 Section 7.4.5:

$x - k * s$
The tolerance limit factor k was determined using the number of measurements performed. The acceptance criteria of the expelled dose is (b) (4) with 99.99% probability content with 95% confidence.
If the $(x - k * s)$ value of the lower tolerance limit was greater than or equal to the minimum dose accuracy (L) 0.6 mL, the test result is noted as "PASS".
 $x - k * s \geq L$
The equation is used to determine the dose accuracy requirements are met for all test conditions and pre-conditioning performed in the design verification.

Results:

Preconditioning		Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result
Standard atmosphere		(b) (4)	60	(b) (4)				Pass
Cool atmosphere		with 99.99% probability content with 95% confidence	60	(b) (4)				Pass
Free fall			60	(b) (4)				Pass
Dry-heat storage			60	(b) (4)				Pass
Cold storage			60	(b) (4)				Pass
Vibration			60	(b) (4)				Pass
Warm atmosphere			60	(b) (4)				Pass
Transport (sub-assemblies)			60	(b) (4)				Pass
Shelf life of sub-assemblies	Accelerated aging corresponding to 1 year		60	(b) (4)				Pass
	Accelerated aging corresponding to 2 years		60	(b) (4)				Pass
	Real time aging, 1 year		60	(b) (4)				Pass
Preconditioning		Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result
	Real time aging, 2 year		60	(b) (4)				Pass
Shelf life of components	Accelerated aging corresponding to 2 years		60	(b) (4)				Pass
	Real time aging, 1 year		60	(b) (4)				Pass

Conclusions/ [Reviewer Comments:](#)

Dose accuracy testing was conducted in accordance with ISO 11608-1. It is to note that the preconditioning temperatures are not as prescribed in the standard. Specifically, the cold and dry-heat storage were done only at (b) (4) respectively as opposed

	to 70°C and -40°C. However, the conducted testing substantiates the labeled storage conditions of the drug. Hence, this is acceptable. The results indicate that the AI conforms to the Standard with no reported failures. This is acceptable.
Acceptable:	☒ Yes ☐ No

Title:	AI: Cap Removal Force
Scope/Objective & Acceptance Criteria:	The cap removal force test conducted for design verification was performed by (b) (4) according to their protocol. This test verifies that the auto-injector cap can be removed without undue force. The cap contains a metal needle shield remover flange that interacts with the PFS rigid needle shield such that the rigid needle shield is removed with the cap. Acceptance Criterion: NMT (b) (4) N
Methods	The test is performed on a qualified and calibrated test equipment with custom fixturing to grip the auto-injector. During the same test procedure for dose accuracy, cap removal force is recorded simultaneously. Un-activated auto-injector samples with the cap on are tested. The cap is gripped with custom fixture mounted on the test equipment, which is then axially displaced until the cap is removed. The force required to remove the cap is recorded with respect to displacement.

Results:	Preconditioning		Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result
	Standard atmosphere		(b) (4)	60					Pass
	Cool atmosphere		with	60					Pass
	Free fall		99.99%	60					Pass
	Dry-heat storage		probability	60					Pass
	Cold storage		content	60					Pass
	Vibration		with 95%	60					Pass
	Warm atmosphere		confidence	60					Pass
	Transport (sub-assemblies)			60					Pass
	Shelf life of final device	Accelerated aging corresponding to 12 months at 5 °C		60					Pass
		Accelerated aging corresponding to 30 months at 5 °C		60					Pass
		Accelerated aging corresponding to 30 months at 5 °C plus 6 months at		60					Pass

	25 °C							
Preconditioning		Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result
Shelf life of sub-assemblies	Accelerated aging corresponding to 1 year		60				(b) (4)	Pass
	Accelerated aging corresponding to 2 years		60					Pass
	Real time aging, 1 year		60					Pass
	Real time aging, 2 year		60					Pass
Shelf life of components	Accelerated aging corresponding to 2 years		60				(b) (4)	Pass
	Real time aging, 1 year		60					Pass
Conclusions/ Reviewer Comments:	(b) (4) and Zealand reported no failures during the testing. Cap removal force testing was done concurrently with dose accuracy testing using a force measurement fixture. This is acceptable. Design Validation: The specification for cap removal force appears very high and the measured forces are much lower. Hence, it is unclear how this specification is validated based on the HF or clinical study. Zealand should provide additional information to validate the specification or lower it. See IR#2 below for details.							
Acceptable:	☐ Yes ☒ No							

Title:	AI: Activation Force
Scope/Objective & Acceptance Criteria:	The activation force test conducted for design verification was performed by (b) (4) according to their protocol. This test verifies that the activation can be performed without undue force. Acceptance criterion: NMT (b) (4) N
Methods	During the same test procedure for dose accuracy, activation force is measured. It is defined as the force required to initiate the activation and is recorded with respect to displacement. The test

is performed on a qualified and calibrated test equipment with custom fixturing to grip the autoinjector. A fixture on the equipment presses on the needle guard which is then axially displaced until the activation is initiated.

Results:

Preconditioning		Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result
Standard atmosphere		(b) (4) with 99.99% probability content with 95% confidence	60				(b) (4)	Pass
Cool atmosphere			60					Pass
Free fall			60					Pass
Dry-heat storage			60					Pass
Cold storage			60					Pass
Vibration			60					Pass
Warm atmosphere			60					Pass
Transport (sub-assemblies)			60					Pass
Shelf life of final device	Accelerated aging corresponding to 12 months at 5 °C		60					Pass

Preconditioning		Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result
	Accelerated aging corresponding to 30 months at 5 °C		60				(b) (4)	Pass
	Accelerated aging corresponding to 30 months at 5 °C plus 6 months at 25 °C		60					Pass
Shelf life of sub-assemblies	Accelerated aging corresponding to 1 year		60					Pass

		Accelerated aging corresponding to 2 years		60	(b) (4)	Pass
		Real time aging, 1 year		60		Pass
		Real time aging, 2 year		60		Pass
	Shelf life of components	Accelerated aging corresponding to 2 years		60		Pass
		Real time aging, 1 year		60		Pass

Conclusions/ Reviewer Comments: Activation force is measured concurrently to dose accuracy testing. The fixture applies an axial force to the activation button until the injection is initiated. No failures were reported by the Sponsor. Hence, this is acceptable.

Acceptable: Yes No

Title: AI: Injection Time

Scope/Objective & Acceptance Criteria: *The injection time test conducted for design verification was performed by (b) (4) according to their protocol. This test verifies that the injection time is within specification.*
 Acceptance criterion: NMT (b) (4) seconds

Methods: *During the same test procedure for dose accuracy, injection time is captured. The test is performed on a qualified test equipment that activates the auto-injector to deliver the dose and that measures the injection time by video frame counting on qualified and calibrated vision control equipment.*

Preconditioning	Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result
Standard atmosphere	(b) (4) seconds with 99.99% probability content	60	(b) (4)	(b) (4)	(b) (4)	(b) (4)	Pass
Cool atmosphere		60					Pass
Free fall		60					Pass
Dry-heat storage		60					Pass
Cold storage		60					Pass
Vibration		60					Pass
Warm atmosphere		60					Pass

	Transport (sub-assemblies)	with 95% confidence	60	(b) (4)				Pass																																				
	Shelf life of sub-assemblies	Accelerated aging corresponding to 1 year	60					Pass																																				
Accelerated aging corresponding to 2 years		60					Pass																																					
<table border="1"> <thead> <tr> <th colspan="2">Preconditioning</th> <th>Acceptance criteria</th> <th>Sample size</th> <th>Average</th> <th>Minimum</th> <th>Maximum</th> <th>Standard deviation</th> <th>Result</th> </tr> </thead> <tbody> <tr> <td rowspan="2"></td> <td>Real time aging, 1 year</td> <td rowspan="4"></td> <td>60</td> <td colspan="3">(b) (4)</td> <td>Pass</td> </tr> <tr> <td>Real time aging, 2 year</td> <td>60</td> <td colspan="3"></td> <td>Pass</td> </tr> <tr> <td rowspan="2">Shelf life of components</td> <td>Accelerated aging corresponding to 2 years</td> <td>60</td> <td colspan="3"></td> <td>Pass</td> </tr> <tr> <td>Real time aging, 1 year</td> <td>60</td> <td colspan="3"></td> <td>Pass</td> </tr> </tbody> </table>									Preconditioning		Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result		Real time aging, 1 year		60	(b) (4)			Pass	Real time aging, 2 year	60				Pass	Shelf life of components	Accelerated aging corresponding to 2 years	60				Pass	Real time aging, 1 year	60				Pass
Preconditioning		Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result																																				
	Real time aging, 1 year		60	(b) (4)			Pass																																					
	Real time aging, 2 year		60				Pass																																					
Shelf life of components	Accelerated aging corresponding to 2 years		60				Pass																																					
	Real time aging, 1 year		60				Pass																																					
Conclusions/ Reviewer Comments:	Testing was done concurrently to dose accuracy measurements by video frame counting using video control equipment. No failures were reported by (b) (4) or Zealand. This is acceptable.																																											
Acceptable:	☒ Yes ☐ No																																											
Title:	AI: Activation resistance force																																											
Scope/Objective & Acceptance Criteria:	<i>The activation resistance force test conducted for design verification was performed by (b) (4) according to their protocol. This test verifies that the threshold for unintended activation is within specification.</i> Acceptance criterion: NLT (b) (4)N																																											
Methods	<i>The test is performed on a qualified and calibrated test equipment with custom fixturing to grip the auto-injector. A fixture on the equipment presses on the needle guard which is then axially displaced. During the same test procedure for dose accuracy, activation resistance force is measured.</i>																																											

Preconditioning		Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result
Standard atmosphere		(b) (4) with 99.99% probability content with 95% confidence	60	(b) (4)				Pass
Cool atmosphere			60					Pass
Free fall			60					Pass
Dry-heat storage			60					Pass
Cold storage			60					Pass
Vibration			60					Pass
Warm atmosphere			60					Pass
Transport (sub-assemblies)			60					Pass
Shelf life of final device	Accelerated aging corresponding to 12 months at 5 °C		60					Pass
	Accelerated aging corresponding to 30 months at 5 °C		60					Pass
	Accelerated aging corresponding to 30 months at 5 °C plus 6 months at 25 °C	60	Pass					
Shelf life of sub-assemblies	Accelerated aging corresponding to 1 year	60	Pass					
	Accelerated aging corresponding to 2 years	60	Pass					
	Real time aging, 1 year	60	Pass					
	Real time aging, 2 year	60	Pass					
Shelf life	Accelerated	60	Pass					

	of components	aging corresponding to 2 years							
		Real time aging, 1 year		60	6	6	7	0.2	Pass
1 The acceptance criteria for this EPR is $\geq$ (b) (4) N. The design verification testing was performed at (b) (4) with a stricter acceptance criteria of (b) (4) N.									
Conclusions/ Reviewer Comments:	The Sponsor identified a minimum activation force of the AI as an EPR. The EPR was verified similarly to activation force by applying an axial force to the AI. No failures were reported by (b) (4) or Zealand. This is acceptable.								
Acceptable:	☒ Yes ☐ No								

Title:	AI: Needle Extension																																																																																								
Scope/Objective & Acceptance Criteria:	The needle extension test conducted for design verification was performed by (b) (4) according to their protocol. This test verifies that the needle extension, i.e. the distance between the needle tip and the needle guard is within specification.																																																																																								
Methods	The test is performed on qualified and calibrated vision control equipment with custom fixturing to grip the auto-injector. During the same test procedure for dose accuracy, needle extension is captured. The test is performed on auto-injector samples that have been activated to deliver the dose but not yet lifted from the surface onto which the device was depressed, thus positioning the needle guard fully retracted and not locked out. Acceptance Criteria: NLT (b) (4), NMT (b) (4)																																																																																								
Results:	<table border="1"> <thead> <tr> <th>Preconditioning</th> <th>Acceptance criteria</th> <th>Sample size</th> <th>Average</th> <th>Minimum</th> <th>Maximum</th> <th>Standard deviation</th> <th>Result</th> </tr> </thead> <tbody> <tr> <td>Standard atmosphere</td> <td>$\leq$ (b) (4) and (b) (4)</td> <td>60</td> <td></td> <td></td> <td></td> <td>(b) (4)</td> <td>Pass</td> </tr> <tr> <td>Cool atmosphere</td> <td>$\leq$ (b) (4)</td> <td>60</td> <td></td> <td></td> <td></td> <td></td> <td>Pass</td> </tr> <tr> <td>Free fall</td> <td>with 99.99% probability</td> <td>60</td> <td></td> <td></td> <td></td> <td></td> <td>Pass</td> </tr> <tr> <td>Dry-heat storage</td> <td>content</td> <td>60</td> <td></td> <td></td> <td></td> <td></td> <td>Pass</td> </tr> <tr> <td>Cold storage</td> <td>with 95% confidence</td> <td>60</td> <td></td> <td></td> <td></td> <td></td> <td>Pass</td> </tr> <tr> <td>Vibration</td> <td></td> <td>60</td> <td></td> <td></td> <td></td> <td></td> <td>Pass</td> </tr> <tr> <td>Warm atmosphere</td> <td></td> <td>60</td> <td></td> <td></td> <td></td> <td></td> <td>Pass</td> </tr> <tr> <td>Transport (sub-assemblies)</td> <td></td> <td>60</td> <td></td> <td></td> <td></td> <td></td> <td>Pass</td> </tr> <tr> <td>Shelf life of final</td> <td>Accelerated aging</td> <td>60</td> <td></td> <td></td> <td></td> <td></td> <td>Pass</td> </tr> </tbody> </table>									Preconditioning	Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result	Standard atmosphere	$\leq$ (b) (4) and (b) (4)	60				(b) (4)	Pass	Cool atmosphere	$\leq$ (b) (4)	60					Pass	Free fall	with 99.99% probability	60					Pass	Dry-heat storage	content	60					Pass	Cold storage	with 95% confidence	60					Pass	Vibration		60					Pass	Warm atmosphere		60					Pass	Transport (sub-assemblies)		60					Pass	Shelf life of final	Accelerated aging	60					Pass
Preconditioning	Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result																																																																																		
Standard atmosphere	$\leq$ (b) (4) and (b) (4)	60				(b) (4)	Pass																																																																																		
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Transport (sub-assemblies)		60					Pass																																																																																		
Shelf life of final	Accelerated aging	60					Pass																																																																																		

	device	corresponding to 12 months at 5 °C			(b) (4)				
		Accelerated aging corresponding to 30 months at 5 °C	60					Pass	
		Accelerated aging corresponding to 30 months at 5 °C plus 6 months at 25 °C	60					Pass	
	Shelf life of sub-assemblies	Accelerated aging corresponding to 1 year	60					Pass	
		Accelerated aging corresponding to 2 years	60					Pass	
	Preconditioning		Acceptance criteria	Sample size	Average	Minimum	Maximum	Standard deviation	Result
		Real time aging, 1 year		60	(b) (4)			Pass	
		Real time aging, 2 year		60				Pass	
	Shelf life of components	Accelerated aging corresponding to 2 years		60				Pass	
		Real time aging, 1 year		60				Pass	
Conclusions/ Reviewer Comments:		Needle length extension was verified on the fully depressed AI during dose accuracy testing. This is acceptable as it represents the needle length during injection. No failures were reported by (b) (4) or Zealand. Hence, this is acceptable.							

Design Validation Information:

The Sponsor provided information regarding the design validation of this performance attribute in 3.2.R Device – Design Validation Summary – Auto-injector. The Sponsor stated that this needle extension was evaluated against the potential risk of intraosseous injection for pediatric patients. The following table was provided (with reference):

Table 4 Percentage of children where a needle may touch the bone

Body weight	Needle length			
	6.5 mm	7 mm	7.5 mm	8 mm
7,5 kg	(b) (4)			
11 kg				
15 kg				

Zealand states that there is no negative clinical impact of reaching the bone of a patient with the needle and that the only side effect may be pain. In addition, an intraosseous injection is unlikely as higher injection forces are needed.

Similar needle extension lengths are typically utilized for subcutaneous injections. For example, AMJEVITA is administered subcutaneously and has a needle extension length of 6 mm ± 1.5 mm and is indicated for patients 15 kg and higher.

The chosen needle extension length appears to be sufficiently validated.

Acceptable:

Yes No

Reviewer Comment

The Sponsor provided raw data of the device verification activities in response to IR#1 in Sequence 008.

Insert Additional Design Verification Table

9.2. Design Verification Review Conclusion

DESIGN VERIFICATION REVIEW CONCLUSION		
Filing Deficiencies: ☒ Yes ☐ No ☐ N/A	Mid-Cycle Deficiencies: ☒ Yes ☐ No ☐ N/A	Final Deficiencies: ☐ Yes ☒ No ☐ N/A
<u>Reviewer Comments</u> There is an outstanding IR regarding device shelf-life/stability data. The Sponsor stated that additional data will be provided by the end of December. As of now, the provided stability data is NOT sufficient to support the claimed shelf-life. Please see IR#9 below for details. UPDATE 1/11/2021: All IRs have been resolved. There are no outstanding deficiencies/information requests.		
CDRH sent Design Verification Deficiency or Interactive Review Questions to the Sponsor: ☒ Yes ☐ No		

	Date Sent: 8/10/2020	Date/Sequence Received: 9/18/2020 Seq. 26
Information Request #2	You stated that you evaluate cap removal force as an essential performance requirement of your proposed auto-injector with an acceptance criterion is $\leq$ (b) (4) N. This specification appears high and your actual test results for cap removal force are much lower (mean test results after preconditioning were between (b) (4) N according to 3.2.R Device - Design Verification Summary - Auto-injector). Due to the large difference between test results and your acceptance criterion, it is unclear how the conducted clinical or human factors study validates the cap removal specification. Provide additional validation data (e.g., literature review) to support your cap removal specification or tighten your specification to be closer to the measured forces.	
Sponsor Response	Zealand Pharma A/S (termed Zealand hereafter) agrees to reduce the auto-injector cap removal force acceptance criterion from $\leq$ (b) (4) N to $\leq$ (b) (4) N. This modified criterion is based on the maximum potential rigid needle shield removal force specified by the syringe supplier ($\leq$ (b) (4) N) plus (b) (4) N to accommodate for any friction imparted by the auto-injector cap to auto-injector body (needle guard) interface. The below-listed documents have been updated to reflect the revised acceptance criterion: 3.2.P.2.4 Container Closure System, 3.2.P.5.1 Specification – Auto-injector, 3.2.P.5.6 Justification of Specifications, 3.2.R Device - Design Verification Summary - Auto-injector, 3.2.R Device - Reliability Performance Report - Auto-injector, 3.2.R Device - Manufacturing Control Strategy – Auto-injector and 3.2.R Device - Overview of Design Control Process – Auto-injector.	
Reviewer Comments	Zealand has lowered the cap removal force specification for the auto-injector to NMT (b) (4) N. While this specification is still higher than the measured values, it is in line with similar injectors. Zealand provided updated device documentation that reflect the new specification. In the updated 3.2.P.5.6 – Justification of Specifications, the Sponsor stated that an additional lower limit for cap removal force is applied to the device (NLT (b) (4) N). However, this specification is not considered an EPR. This is acceptable.	

Response Adequate: Yes No, See IR # Sent on Click or tap to enter a date.

	Date Sent: 8/10/2020	Date/Sequence Received: 9/18/2020 Seq. 26
Information Request #3	In 3.2.R “Device – Design Verification Summary – Pre-filled Safety Syringe” and 3.2.R “Device – Design Verification Summary – Auto-injector”, you stated that accelerated aging was done for 52 days at 45°C with subsequent aging for 6 months at 25°C for device performance testing. However, it is unclear how these aging parameters are representative of your proposed shelf-life of 3 years when stored at (b) (4) C with the option to store the product for 1 year at 25°C. Provide a rationale of how your accelerated aging with subsequent 6 months storage at 25°C supports your shelf-life claim or conduct additional testing of devices that underwent accelerated and/or real-time aging representative of 2 years at 5°C and 1 year at 25°C.	
Sponsor Response	(b) (4) This has now been corrected, and the applied accelerated aging preconditioning has been clarified throughout 3.2.R Device - Design Verification Summary – Pre-filled Safety Syringe and 3.2.R Device - Design Verification Summary – Auto-injector. (b) (4)	

	(b) (4) All design verification and reliability testing EPR results comply with the acceptance criteria, thereby supporting the proposed shelf life of “36 months at 2-8°C, including a single period of maximum 12 months at room temperature up to 25°C”.
Reviewer Comments	The Sponsor’s response clarifies how accelerated aging was calculated (b) (4) (b) (4) (b) (4). Based on the provided aging parameters, a shelf-life of (b) (4) years may be supported with the provided data. A follow-on deficiency will be issued to the Sponsor.
Response Adequate:	☐ Yes ☒ No, See IR # Sent on Click or tap to enter a date.

Follow-On Deficiency	Date Sent: 10/9/2020	Date/Sequence Received: 10/26/2020 Seq 32
Information Request #8	In your response to Question 2 of our information request dated August 27, 2020, you stated that, when calculating your accelerated aging time, you used an aging factor (b) (4) instead of the commonly utilized Q₁₀ of 2.0. Per ASTM F1980-16 <i>Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices</i>, a more aggressive reaction rate coefficient may only be chosen if the system under investigation is sufficiently characterized in the literature. We are not aware of available information that would substantiate use of a more aggressive aging factor. Re-calculate the shelf-life of the device constituent parts of your combination product using a Q₁₀ factor of 2.0. Alternatively, provide a scientific justification and literature data that would support the use of an aging factor (b) (4) for your device.	
Sponsor Response	Response to question 1	

Zealand would like to clarify that the calculation using the aging factor (b) (4) provided in our response to question 2 provided in the amendment dated September 18, 2020 was applied for the pre-filled safety syringe.

For the auto-injector a similar approach using the full Arrhenius equation and actual determined activation energies of the auto-injector materials was applied. Both approaches are considered consistent with ASTM F1980-16 Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices.

Scientific justifications of the shelf life of the device constituent parts of the dasiglucagon pre- filled safety syringe and auto-injector presentations is provided below.

(b) (4)

	Thus, the proposed shelf life of the device constituent parts of the dasiglucagon auto-injector is justified.
Reviewer Comments	The Sponsor used accelerated aging parameters that are less conservative as what is recommended in ASTM F1980. For the AI, accelerated aging parameters were based on <i>internal</i> test data that was not available online and it is unclear if this data was peer reviewed. In addition, it is unclear how the individual degradation reactions of materials is representative of the degradation of the device as a whole. Hence, the response for the AI is <u>not acceptable</u>. For the PFS configuration, the Sponsor provided data after real-time aging that indicates that the device stays within the performance specifications throughout the intended shelf-life. This is acceptable.
Response Adequate:	☐ Yes ☒ No, See IR #9 Sent on 11/10/2020

Follow-On Deficiency	Date Sent: 11/10/2020	Date/Sequence Received: 12/22/2020 Seq 40
Information Request #9	In your response to our information request dated October 13, 2020, you stated that your accelerated aging parameters for the auto-injector are based on activation energies of degradation reactions (e.g., oxidation, hydrolysis, etc.) of some materials used in the construction of the device. However, this assessment does not cover all materials or components used in the manufacture of the device and does not consider how the system is projected to age as a whole. Per ASTM F1980-16, the <i>system under investigation</i> should be characterized in the literature to justify less conservative accelerated aging parameters. Furthermore, as your device is intended for emergency use, conservative preconditioning parameters should be chosen to ensure that your device performs adequately throughout its shelf-life. We disagree that your aging protocol provides a conservative means of ensuring that your device performs adequately throughout the intended shelf-life. Provide ONE of the following for your proposed auto-injector configuration: - Recalculate your shelf-life using a Q₁₀ factor of 2.0; OR - Provide additional testing of devices that underwent accelerated aging (using a Q₁₀ factor of 2.0) representative of the device (b) (4) OR - Provide performance data from real-time aged devices stored (b) (4) (b) (4) to support your shelf-life claim.	
Sponsor Response	Zealand Pharma will provide additional performance testing results of assembled auto-injectors that have been aged by accelerated aging time corresponding to the proposed shelf life (b) (4) when applying an aging factor (Q₁₀) of 2.0 (i.e. option b). Specifically, samples of auto-injector batches V0159D1A and V0159D1B which have already been aged by accelerated aging at 45°C in relation to reliability performance testing (see 3.2.R Device – Reliability Performance Report - Auto-injector) is currently subjected to additional accelerated aging, in order to reach a cumulative accelerated aging time equivalent to real time aging (b) (4) The aging time will be calculated using the formula	

provided in ASTM F1980-16 applying the conservative acceleration factor (Q10) of 2.0 as requested by the Agency; and thus is being representative for the proposed end of shelf life of the auto-injector.

The performance of the aged auto-injectors will be tested with respect to essential performance requirements and the least reliable base case after the accelerated aging time.

The results from the additional performance testing representing the proposed shelf life applying a Q10 factor of 2.0 in the calculation of the accelerated aging time will be provided at latest by end of December 2020. The submission will comprise updated sections 3.2.R Device – Reliability Performance Report - Auto-injector and 3.2.R Device – Design Verification Summary – Auto-injector, integrating the additional performance data.

UPDATE SEQUENCE 40:

Zealand Pharma has conducted additional performance testing of assembled auto-injectors that have been aged by a combination of accelerated and real time aging corresponding to the proposed shelf life, applying an aging factor (Q10) of 2.0 as requested by the Agency (i.e. option B).

(b) (4)

The performance testing conducted on the aged samples is summarized in subsection 1.2.

(b) (4)



1.2 Performance testing

The performance of the aged auto-injectors has been tested with respect to essential performance requirements and the least reliable base case after combined accelerated and real time aging combined with various pre-conditioning test scenarios (see [Figure 1](#)), similarly to previous reliability testing.

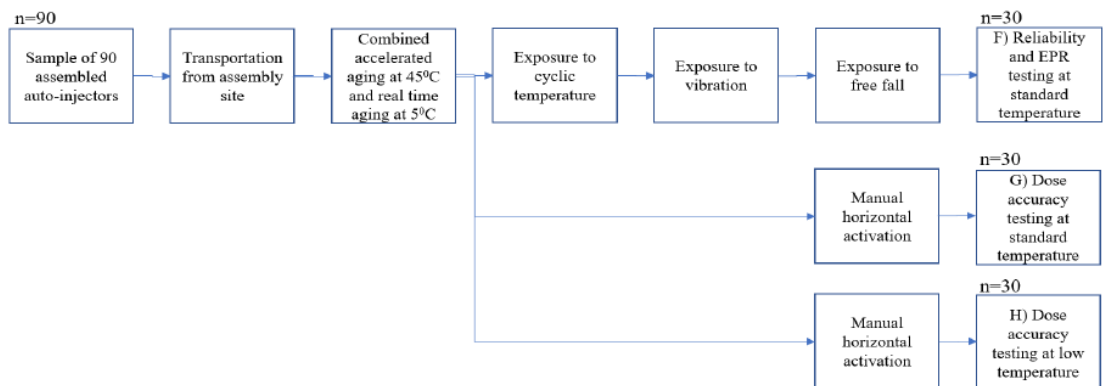


Figure 1 Overview of additional reliability testing performed using samples aged by combined accelerated and real time aging

A summary of the additional reliability performance testing conducted including results and conclusions are provided in 3.2.R Device – Reliability Performance Report - Auto-injector. The conclusions have also been integrated in 3.2.R Device – Design Verification Summary – Auto-injector, subsection 2.5.

	In summary, all essential performance requirements passed the tests at a 95% confidence level and 99.99% probability content. The least reliable base case (i.e. completion of rotator movement measured as ‘Dimension AB’) fulfilled the performance requirement based on the 95% confidence and 99.99985% probability content. All auto-injectors fired. Thus, the reliability performance testing performed on samples aged at combined accelerated aging and real time storage confirms that the essential performance requirements and the ability to fire requirements are maintained throughout the proposed shelf-life of the auto-injector. 1.3 Conclusion The proposed shelf life of the device constituent parts of the dasiglucagon auto-injector is confirmed by the results of the additional reliability performance testing performed on auto-injector samples aged to the proposed end of shelf life of 36 months at (b) (4) C (including a period of 12 months at room temperature up to 25°C), also when using a Q10 factor of 2.0.																																						
Reviewer Comments	The Sponsor has provided additional data after accelerated aging of the autoinjector. Aging was done in conformance with ASTM F1980 using a conservative (recommended) Q₁₀ value of 2.0. The aging parameters were reviewed and found acceptable. The following test results were provided by the Sponsor after aging and various preconditioning: Aging, Simulated Shipping and Handling (cyclic temperatures, vibration, free fall): Table 19 Reliability performance test results (Exposure F in Figure 2) <table border="1" data-bbox="410 1073 1550 1545"> <thead> <tr> <th>Test</th> <th>k-factor</th> <th>Mean</th> <th>SD</th> <th>Tolerance interval limit</th> <th>Acceptance criteria</th> <th>PASS/FAIL</th> </tr> </thead> <tbody> <tr> <td>Dose accuracy</td> <td colspan="4" rowspan="7" style="background-color: #cccccc;">(b) (4)</td> <td>(b) (4)</td> <td>PASS</td> </tr> <tr> <td>Cap removal force¹</td> <td>PASS</td> </tr> <tr> <td>Injection time</td> <td>PASS</td> </tr> <tr> <td>Activation force</td> <td>PASS</td> </tr> <tr> <td>Activation resistance force</td> <td>PASS</td> </tr> <tr> <td>Needle extension</td> <td>PASS</td> </tr> </tbody> </table> Aging, injection at standard temperature: Table 20 Reliability performance test results (Exposure G in Figure 2) <table border="1" data-bbox="410 1675 1550 1822"> <thead> <tr> <th>Test</th> <th>k-factor</th> <th>Mean</th> <th>SD</th> <th>Tolerance interval limit</th> <th>Acceptance criteria</th> <th>PASS/FAIL</th> </tr> </thead> <tbody> <tr> <td>Dose accuracy</td> <td colspan="4" rowspan="1" style="background-color: #cccccc;">(b) (4)</td> <td>(b) (4)</td> <td>PASS</td> </tr> </tbody> </table> Aging, injection at low temperatures: Table 21 Reliability performance test results (Exposure H in Figure 2)	Test	k-factor	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL	Dose accuracy	(b) (4)				(b) (4)	PASS	Cap removal force ¹	PASS	Injection time	PASS	Activation force	PASS	Activation resistance force	PASS	Needle extension	PASS	Test	k-factor	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL	Dose accuracy	(b) (4)				(b) (4)	PASS
Test	k-factor	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL																																	
Dose accuracy	(b) (4)				(b) (4)	PASS																																	
Cap removal force ¹					PASS																																		
Injection time					PASS																																		
Activation force					PASS																																		
Activation resistance force					PASS																																		
Needle extension					PASS																																		
Test					k-factor	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL																													
Dose accuracy	(b) (4)				(b) (4)	PASS																																	

	Test	k-factor	Mean	SD	Tolerance interval limit	Acceptance criteria	PASS/FAIL	
	Dose accuracy	(b) (4)					(b) (4)	PASS
Each set of testing was carried out with a sample size of 30 for a total sample size of 90 for dose accuracy testing. The Sponsor stated that all tested AIs fired and that the data is within the predefined acceptance criteria. Looking at the results, no performance attribute showed a significant change compared to testing with non-aged devices. The Sponsor has adequately addressed this IR.								
Response Adequate:	☒ Yes ☐ No, See IR # Sent on Click or tap to enter a date.							



(b) (4)

9.3. Device Safety Assessment (Sterilization and Biocompatibility)

9.3.1. Sterilization

(b) (4) A review of the sterilization of components, (b) (4) filling, and container closure integrity testing (CCIT) is deferred to the relevant review division in CDER.

9.3.2. *Biocompatibility*

This biocompatibility review/assessment is limited to the non-fluid path components of the device. An pre-clinical assessment of the fluid path components and drug product is deferred to the relevant review division in CDER.

The device components that require a biocompatibility assessment are the AI subassemblies and the plunger component of the syringe. Other components (i.e., the syringe barrel, needle, etc.) are part of the fluid path and will not be evaluated as part of this memo.

PFS:

The plunger is made by (b) (4) and has a master file in DMF (b) (4) (b) (4) and has limited contact with intact skin. This contact classification warrants an assessment of the cytotoxicity, sensitization, and irritation endpoints per the 2016 FDA biocompatibility guidance. Zealand is leveraging the biocompatibility information from the DMF. The DMF contains a summary of cytotoxicity testing of the polymer:

Table 1-5: Summary of *In Vitro* Bioreactivity Data

Test	Acceptance Limit	Trial	Control Samples		Test Sample	Pass/Fail
			Negative	Positive		
MEM Elution Test	NMT ¹	(b) (4)			(b) (4)	Pass
						Pass
						Pass

Notes:

1. NMT = Not More Than

The data indicates that the plunger material is not cytotoxic. While testing per ISO 10993-10 was not provided, the plunger material is commonly used in prefilled syringes and has a long history of safe use. Furthermore, the plunger only contacts intact skin for a limited period of time (< 24 hours). As the history of this material is well established for intact skin contact and the fact that a patient will only have brief contact with the component, no additional biocompatibility data is needed to support the PFS.

AI:

Biocompatibility testing of the AI subassemblies was conducted by (b) (4) and information was provided in MAF (b) (4). Similar to the PFS plunger, the AI components are only in contact with intact skin for a limited time (<24 hours). The following biocompatibility test summaries were provided:

Component		Material	Color
Front Subassembly	Body	(b) (4)	
	Needle Cover		
	Front Cap		

	Front Cap Cover	(b) (4)
	RNS Remover	
Rear Subassembly	Rear Body	

Test Item	Reference	Acceptance Criteria	Result
Cytotoxicity	ISO 10993-5	(b) (4)	Pass
Test Item	Reference	Acceptance Criteria	Result
Irritation	ISO 10993-10	(b) (4)	Pass
Skin Sensitization	ISO 10993-10	(b) (4)	Pass

Although full test reports were not provided, the provided summaries are sufficient to support the use of the auto-injector given the contact classification of its components. The device is made from materials that have a long history of safe use in medical devices (ABS, PC, POM, and stainless steel) and the test summaries indicate that the device passed all biological testing. A request for additional biocompatibility information or full test reports is not warranted given the intended use of the device and the materials of construction. Hence, the biocompatibility evaluation is acceptable.

9.4. Discipline Specific Sub-Consulted Review Summary

- No Additional Discipline Specific Sub-Consults were requested
- The following additional Discipline Specific Sub-Consults were requested:

10. CLINICAL VALIDATION REVIEW

10.1. Review of Clinical Studies Clinical Studies

- There is no device related clinical studies for review
 There are clinical studies for review

This information was obtained from the following [documents](#): Seq 001 5.3.5.1: ZP4207-16137 and ZP4207-17145

Study Name	A Phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®
Study Type	Phase 3
Objectives/Endpoints	Primary: - To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous (SC) 0.6 mg dose administered to patients with T1DM with insulin-induced hypoglycemia. Secondary: - To compare the glycemc response observed after dasiglucagon with that of GlucaGen®.
Drug/Device Studied	0.6 mL PFS
Number and Type of Subjects	Planned: 176 patients were planned to be randomized to have 156 patients (78 patients in the dasiglucagon group and 39 patients in each of the placebo and GlucaGen® groups) complete the treatment visit. Analyzed: Total of 170 patients were eligible and randomized to dasiglucagon, placebo or GlucaGen® treatment. One hundred and sixty-eight (168) patients completed the trial. Key inclusion criteria: - Male and female patients with type 1 diabetes mellitus treated with insulin for at least one year, diagnostic criteria as defined by the American Diabetes Association. - Stable insulin treatment 30 days prior to screening, defined as no more than a 10-unit daily variation in total daily insulin dose. - Hemoglobin A1c <10%. - Aged between 18 and 75 years, both inclusive. Key exclusion criteria: Patients previously treated with dasiglucagon, who had a history of anaphylaxis, pheochromocytoma, insulinoma, or congestive heart failure, hypoglycemic events associated with seizures in the year before screening, active malignancy within the last 5 years, clinically significant illness within 4 weeks before screening, surgery or trauma with significant blood loss within 4 weeks before screening, and clinically abnormal electrocardiogram (ECG) at screening were excluded from the trial. The use of certain medications including beta-blockers, indomethacin, warfarin and anticholinergic drugs also prevented patients from taking part in the trial. In total, 235 patients were screened, of which 170 patients were eligible and randomized to

	dasiglucagon, placebo or GlucaGen® treatment. One hundred and sixty-eight (168) patients received trial drug, comprising the safety and full analysis sets. All 168 dosed patients completed the trial. Two (2) patients in the dasiglucagon group discontinued prior to being treated (due to withdrawal of consent and AE, respectively). The majority of the patients in this trial were male (63%) and white (92%) and non-Hispanic (96%). The overall mean (SD) age was 39.1 (12.18) years. The demographic and baseline characteristics were similar across the three treatment groups.
Brief description of protocol	This trial was a global, multicenter, randomized, parallel-group, double-blind, clinical trial confirming the efficacy and safety of dasiglucagon for treatment of hypoglycemia in patients with T1DM. The patients were randomized 2:1:1 to receive a single SC dose of 0.6 mg dasiglucagon, placebo, or 1 mg GlucaGen® following a hypoglycemic clamp procedure. Patients were followed for at least 28 days after receiving treatment. The trial was conducted in Germany, Austria, Canada and the United States.
Results	• The proportion of patients reporting AEs was 80%, 74% and 33% in the dasiglucagon, GlucaGen® and placebo treatment groups, respectively. • The most frequently reported AEs in both the dasiglucagon and GlucaGen® groups were nausea (55% and 53% of patients, respectively, and 2% in the placebo group), hypoglycemia (28% and 21%, respectively, and 12% for placebo), vomiting (23% and 21%, respectively, and 2% for placebo) and headache (12% and 12%, respectively, and 5% for placebo). Nausea, vomiting and headache were also the most frequently reported AEs assessed as possibly or probably related by the investigator. • All AEs were mild or moderate except for non-serious severe AEs of nausea and vomiting in one patient in the GlucaGen® group. • None of the reported events of hypoglycemia were evaluated by the investigator as related to investigational product treatment, and all were mild AEs from which the patients recovered. Furthermore, only two events of hypoglycemia occurred on the dosing day after dosing (one with dasiglucagon and one with GlucaGen®, both approximately 6 hours after dosing). • No serious AEs, deaths, or treatment-emergent AEs (TEAEs) leading to discontinuation occurred. • No patients in the active treatment arms received rescue infusion of IV glucose. • Local tolerability assessments showed few events: 2 separate events in 2 patients in the dasiglucagon group (pain on palpation and redness), 2 events in 2 patients in the placebo group (redness in both patients) and 3 events in 3 patients in the GlucaGen® group (redness in 2 patients and edema in 1 patient). • A total of 3 AEs of special interest were observed in 3 separate patients: bradycardia and presyncope in the dasiglucagon group, and orthostatic hypotension in the GlucaGen® group. All 3 AEs of special interest were transient and resolved. • No clinically significant changes were observed for laboratory parameters • No clinically significant changes were observed in vital signs. Small increases in pulse occurred among patients on active treatment; no changes in mean blood pressure were observed. • No clinically significant changes were observed in ECG parameters, including QTcF assessment.

	One patient was positive for anti-dasiglucagon antibodies following dasiglucagon treatment. The antibody titer was 24.4 and antibodies did not cross-react with glucagon. At subsequent follow up, antibody titers were negative for the patient.
Device Related Comments	No device specific comments were found in the protocol.
Reviewer Comments	There were no deaths or serious adverse events in the study. AEs included gastrointestinal symptoms (nausea, vomiting), headaches, and injection site erythema (4 events total). The injection site erythema was reported in the placebo group and the dasiglucagon group. It is unlikely that the injection site erythema was caused by the device and was found in both the placebo and the drug group.
Reviewer Conclusion	No adverse events were reported that would indicate an issue with the device.

Study Name	A randomized, double-blind, parallel-group trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes
Study Type	Phase 3
Objectives/Endpoints	There was one primary objective in this study: To demonstrate superiority of dasiglucagon compared to placebo following a single subcutaneous (SC) 0.6 mg dose administered to subjects with type 1 diabetes mellitus (T1DM) with insulin-induced hypoglycemia. - The primary endpoint was time to plasma glucose recovery, defined as first increase in plasma glucose of ≥ 20 mg/dL (1.1 mmol/L) from baseline without administration of rescue intravenous (IV) glucose. - Key secondary endpoints included plasma glucose recovery within 30 minutes, within 20 minutes, within 15 minutes, and within 10 minutes after trial drug injection without administration of rescue IV glucose, and plasma glucose changes from baseline at 30 minutes, at 20 minutes, at 15 minutes, and at 10 minutes after trial drug injection or at the time of rescue. There was one secondary objective in this study: To evaluate the safety, immunogenicity and pharmacokinetics (PK) of dasiglucagon following a single SC dose administered to subjects with T1DM with insulin-induced hypoglycemia as compared to placebo. Endpoints were: - Clinical efficacy (pharmacodynamic [PD]) endpoints were time to first plasma glucose concentration ≥ 70 mg/dL (3.9 mmol/L) without administration of rescue IV glucose, and plasma glucose response as area under the effect curve (AUE) above baseline from time zero to 30 minutes, AUE_{0-30min}. - Exposure (PK) endpoints were area under the drug concentration curve (AUC) from time zero to 90 minutes and 120 minutes, AUC_{0-90min} and AUC_{0-120min} (AUC_{0-120min} was added to the SAP prior to unblinding), maximum plasma drug concentration (C_{max}), and time to maximum plasma drug concentration (t_{max}). - Safety endpoints were adverse events (AEs), clinical laboratory assessments (biochemistry, hematology, urinalysis), local tolerability, number of rescue infusions of IV glucose after trial drug administration, and time to first rescue infusion of IV glucose after trial drug administration. - The immunogenicity endpoint was occurrence of anti-drug antibodies (ADAs).

	There was one tertiary/exploratory objective in this study: To serve as a basis for explaining or supporting findings of primary analyses and for suggesting further hypotheses for later research. Endpoints were: •Plasma glucose concentration ≥ 70 mg/dL or increase of ≥ 20 mg/dL (1.1 mmol/L) within 30 minutes after trial drug injection without administration of rescue IV glucose •Plasma insulin increase as area under the curve above baseline from time zero to 60 minutes, AUC_{0-60min}.
Drug/Device Studied	0.6 mL dose in AI administered by clinical staff.
Number and Type of Subjects	Number of Subjects (planned and analyzed): <i>Planned:</i> The study planned to randomize up to a total of 46 subjects to have 40 subjects complete the dosing visit (Visit 2), with 30 subjects completing the dosing visit in the dasiglucagon group and 10 subjects completing the dosing visit in the placebo group. <i>Analyzed:</i> The study enrolled 68 subjects, 45 of which were randomized to either dasiglucagon (34 subjects) or placebo (11 subjects). Of these, 44 subjects were included in the Safety population (34 subjects randomized to dasiglucagon and 10 subjects randomized to placebo), 44 subjects were included in the Full Analysis Set (FAS) (34 subjects randomized to dasiglucagon and 10 subjects randomized to placebo), and 43 subjects were included in the Per-Protocol (PP) population (33 subjects randomized to dasiglucagon and 10 subjects randomized to placebo). Main Criteria for Inclusion and Exclusion: <i>Key Inclusion Criteria:</i> •Male or female subjects with T1DM treated with insulin for at least one year; diagnostic criteria as defined by the American Diabetes Association. •Treated with insulin for T1DM for at least 1 year and with stable insulin treatment (defined as no more than a 10-unit daily variation in total daily insulin dose) 30 days prior to screening. •Hemoglobin A_{1c} <10%. •Aged between 18 and 75 years, both inclusive. <i>Key Exclusion Criteria:</i> Key exclusion criteria comprised: •Previous participation in a clinical trial within the dasiglucagon in the rescue treatment of hypoglycemia program. •Known or suspected allergy to trial drug(s) or related products. •History of anaphylaxis or symptoms of severe systemic allergy (such as angioedema). •Previous participation in this trial. Participation being defined by signing the informed consent document.
Brief description of protocol	This trial was a multi-center, randomized, parallel-group, double-blind study conducted in adult subjects with T1DM. The study drugs, dasiglucagon or placebo, were administered to the subjects in a randomized manner. Each subject underwent a screening visit (Visit 1) up to 30 days prior to randomization and dosing, an overnight

	in-house visit, where dosing took place (Visit 2), and a follow-up visit (Visit 3). The study design is shown in Figure 2-1. Subjects checked in to the site on Day -1 for an overnight stay. On Day 1 (Visit 2), after an overnight fast, subjects were randomized to receive a single SC dose of dasiglucagon (0.6 mg) or matching placebo. During the dosing visit (Visit 2), hypoglycemia was induced by a fast-acting insulin glulisine (Apidra®) infusion, and trial drug was administered when the subject's plasma glucose level was 45-60 mg/dL (2.5-3.3 mmol/L). A rescue glucose infusion could be administered to ameliorate persistent hypoglycemia. During the insulin-induced hypoglycemia, PK and PD samples were drawn, and plasma glucose levels were monitored closely at site for safety reasons. Clinical laboratory testing, vital sign measurements, physical examinations, local tolerability, and 12-lead electrocardiograms (ECGs) were performed at pre-specified timepoints. Reported or observed AEs were recorded throughout the study. Samples were obtained for immunogenicity testing.
Results	<i>Safety Results:</i> •Any TEAE was reported by 24 subjects (70.6%) who received dasiglucagon and 3 subjects (30%) who received placebo. •The most frequently reported TEAEs included nausea (61.8% of subjects who received dasiglucagon; 10% of subjects who received placebo), vomiting (29.4% of subjects who received dasiglucagon), hypoglycemia (17.6% of subjects who received dasiglucagon; 20% of subjects who received placebo), and headache (11.8% of subjects who received dasiglucagon). •The most frequently reported TEAEs that were considered possibly or probably related to the study drug included nausea (61.8% of subjects who received dasiglucagon and 10% of subjects who received placebo) and vomiting (29.4% of subjects who received dasiglucagon). •Of the 29 hypoglycemic events recorded in the dasiglucagon group, 25 events were recorded for two subjects. None of the events were considered possibly or probably related to the study drug, and only 1 event in the dasiglucagon group and 2 events in the placebo group occurred on the dosing day. The majority of hypoglycemic events were mild. •No subjects discontinued the study due to an AE. •There were no SAEs, AESIs or deaths in this study. •There were no clinically significant changes in laboratory evaluations, vital signs, 12-lead ECG measurements, or physical examinations. •Injection site reactions were few and transient. •There was no significant difference in the incidence of rescue infusion of IV glucose after trial drug administration or time to first rescue infusion of IV glucose after trial drug administration between subjects that received dasiglucagon or placebo. •No subjects tested positive for antibodies against dasiglucagon.
Device Related Comments	No device specific comments were found in the protocol.
Reviewer Comments	2 subjects (5.9%) noted injection site pain/injection site erythema. No severe AE was related to the device. No other safety events were reported that could be related to the device.
Reviewer Conclusion	No adverse events were reported that would indicate an issue with the device.

Reviewer Comment

All information requests regarding design verification were resolved during the review.

10.2. Clinical Validation Review Conclusion

CLINICAL VALIDATION REVIEW CONCLUSION		
Filing Deficiencies: ☐ Yes ☒ No ☐ N/A	Mid-Cycle Deficiencies: ☐ Yes ☒ No ☐ N/A	Final Deficiencies: ☐ Yes ☒ No ☐ N/A
<u>Reviewer Comments</u> Based on the two studies cited above, there were no specific adverse events that could be directly linked to the syringe or auto-injector. The Sponsor stated that the to-be-marketed auto-injector and pre-filled syringe were used in the study. (b) (4)		
CDRH sent Clinical Validation Deficiencies or Interactive Review Questions to the Sponsor: ☐ Yes ☒ No		

11. HUMAN FACTORS VALIDATION REVIEW

CDRH Human Factors Review conducted	☐
Human Factors Review deferred to DMEPA	☒

12. FACILITIES & QUALITY SYSTEMS

12.1. Facility Inspection Report Review

CDRH Facilities Inspection Review conducted	☐
CDRH Facilities Inspection Review was not conducted	☒

12.2. Quality Systems Documentation Review

CDRH Quality Systems Documentation Review conducted	☒
CDRH Quality Systems Documentation Review was not conducted	☐

12.2.1. Description of the Device Manufacturing Process

Summary of Manufacturing Process / Production Flow



(b) (4)



Reviewer Comments

The Sponsor provided a full description of the drug and device manufacturing processes. This is acceptable.

Device Manufacturing Process Conclusion

The Sponsor provided adequate information for the summary of the manufacturing process / production flow.

Yes

No

12.2.2. cGMP Review

Does Sponsor have all elements of their GMP compliance approach included in submission:

What Quality System did the Sponsor choose:

v05.02.2019

- Device** QSR-based
- Drug cGMP-Based Streamline –
- Stream-line Both ([no streamlined approach](#))

21 CFR 820.20 Summary of Management Responsibility	Firm(s): Zealand, (b) (4) Rechon	Reviewer Discussion – Zealand described its management controls in 3.2.P.3.3. Zealand implements the drug cGMP streamlined approach and is responsible for the release and overall quality of the final product. Rechon has implemented a drug based cGMP system and (b) (4) has a device based QS GMP system. Figure 1 CGMP systems at respective sites of the manufacturing process Zealand stated that management responsibilities are described in SOP-0001 and management reviews are periodically conducted as described in SOP-0217. Zealand Pharma Corporate Management defines quality objectives based on the results of conducted management reviews. Records of management review meetings are maintained electronically. Zealand maintains an independent Quality Assurance department that reports to a member of the Corporate Management with functional reporting to the CEO of Zealand Pharma. The head of quality is the appointed management representative. Employee training is provided based on their job description and records of job descriptions, training documents, and CVs are kept per SOP-0101. Overall, Zealand has sufficiently summarized its management controls. The overall management structure is described, and a management representative is identified. Individual SOPs are references to indicate that sufficient structure is in place at the firms.

21 CFR 820.30 Summary of Design Controls	Firm(s): Zealand, Rechon, (b) (4)	Reviewer Discussion – Reviewed in detail in Section 7
21 CFR 820.50 Summary of Purchasing Controls	Firm(s): Zealand, Rechon, (b) (4)	Reviewer Discussion – Information regarding purchasing controls are provided in 3.2.P.3.3. Evaluations of vendors is done based on the criticality of their service. Vendor assessment reports are documented, and criteria include technical, quality, financial assessment, and a technical visit. (b) (4) Overall, sufficient summary information was provided to ensure that adequate purchasing controls are in place at the firm. Zealand conducts vendor audits and has agreement in place to govern the change controls and CAPAs.
21 CFR 820.100 Summary of Corrective and Preventive Actions	Firm(s):	Reviewer Discussion – Reviewed in Section 12.2.3 .
21 CFR 820.170 Summary of Installation	Firm(s):	Reviewer Discussion – N/A
21 CFR 820.200 Summary Servicing	Firm(s):	Reviewer Discussion – N/A
Subpart F – Identification and Traceability	Firm(s):	Reviewer Discussion – N/A
Subpart G – Production and Process Controls	Firm(s):	Reviewer Discussion – Reviewed in Section 12.3
Subpart H – Acceptance Activities	Firm(s):	Reviewer Discussion – N/A
Subpart I – Nonconforming Product	Firm(s):	Reviewer Discussion – N/A

Subpart K – Labeling and Packaging Controls	Firm(s):	Reviewer Discussion – N/A
Subpart L – Handling, Storage, Distribution	Firm(s):	Reviewer Discussion – N/A
Subpart M – Records	Firm(s):	Reviewer Discussion – N/A
Subpart O – Statistical Techniques	Firm(s):	Reviewer Discussion – N/A

Reviewer Comments

Zealand has implemented a drug based cGMP streamlined system and indicated compliance with the relevant callouts (820.20, .30, .50, and .100). See below for a review of the CAPA system.

Reviewer’s Note: CDER indicated that a PAI will not be conducted per current guidelines due the public health emergency. A CDER 704a desk review will be conducted instead of Rechon Life Science and (b) (4) will receive a post approval inspection. CDRH will request additional documents as part of the 704a including process validation documents for auto-injector assembly and performance testing.

GMP Compliance Summary Conclusion

The Sponsor provided adequate summary information about the GMP compliance activities	☒ Yes	☐ No

12.2.3. Corrective and Preventive Action Review

The Sponsor provided the following information with regards to corrective and preventive actions:

Zealand Pharma quality management system has established procedures listed in [Table 4](#) in place to ensure sufficient investigation, assessment, and correction for potential quality problems regarding processes and products internally at Zealand Pharma in accordance with 21 CFR Part 820.100. The listed procedures encompass how to act to unforeseeable quality events, both process and product related.

Table 4 Standard operating procedures for 820.100 compliance

Procedure number	Procedure title
SOP-0068	Deviations and CAPA
SOP-0087	Regulatory Inspections
SOP-0155	Quality Events
SOP-0184	Complaint Handlings
SOP-0190	Handling of change requests and deviations from CMOs
SOP-0201	Quality Risk Management

SOP-0217	Quality Management Review	
(b) (4)		

The following table reflects whether the Sponsor addressed the required elements of corrective and preventive action controls:

CAPA Procedure Required Elements	Present
Procedures include requirements to analyze processes, work operations, concessions, quality audit reports, quality records, service records, complaints, returned product, and other sources of quality data to identify existing and potential causes of nonconforming product, or other quality problems.	Yes
Procedures include review and disposition process of nonconforming product, including documentation of disposition. Documentation shall include the justification for use of nonconforming product and the signature of the individual(s) authorizing the use.	Yes
Procedures include appropriate statistical analysis of these quality data to detect recurring quality problems	Yes
Investigations into the cause of nonconformities relating to product, processes, and the quality system	Yes
Includes requirements for identification and implementation of actions needed to correct and prevent recurrence of nonconformities and other quality problems	Yes
Verification or validation of the corrective and preventive actions taken to ensure that such action is effective and does not adversely affect the finished device	Yes
Each manufacturer shall establish and maintain procedures for rework, to include retesting and reevaluation of the nonconforming product after rework, to ensure that the product meets its current approved specifications	Yes

Describes requirements for implementing and recording changes in methods and procedures needed to correct and prevent identified quality problems	Yes
Ensures that information related to quality problems or nonconforming product is disseminated to those directly responsible for assuring the quality of such product or the prevention of such problems	Yes
Submits relevant information on identified quality problems, as well as corrective and preventive actions, for management review	Yes
Requires documentation of all CAPA activities	Yes

Reviewer Comments

Please see the review of response to IR#5 below for a review of the CAPA system.

CAPA Conclusion

The Sponsor provided adequate information for corrective and preventive actions.	☒ Yes	☐ No

12.3. Control Strategy Review

The Sponsor provided the following control strategy information regarding the EPRs of the device constituents:

Essential Performance Requirements Control Strategy Table

* The proposed acceptance criteria for the EPR may be tighter than the design input and should be assessed for adequate quality control/ Sampling Plan (Sampling plan may be review issue depending on the product (e.g. emergency-use)

Essential Performance Requirements	Control Strategy Description - The Sponsor provided the following description of how the essential performance requirements of the combination product are controlled (b) (4)	Acceptable (Y/N/NA)
PFSS		
Deliverable volume	(b) (4) ISO 3951-1	Y
Break loose force	(b) (4) ISO 3951-1	Y
Glide force	(b) (4) ISO 3951-1	Y
AI		
Dose accuracy	(b) (4) ISO 3951-1	Y

Cap removal force	(b) (4)	Y
	ISO 3951-1	
Injection time	(b) (4)	Y
	ISO 3951-1	
Activation force	(b) (4)	Y
	ISO 3951-1	
Activation resistance force	(b) (4)	Y
	ISO 3951-1	
Needle extension	(b) (4)	Y
	ISO 3951-1	

Reviewer Comments

The control strategy appears to be acceptable. (b) (4)

(b) (4)

(b) (4) Overall, this control strategy appears appropriate to ensure that the devices meet their EPRs at release.

Control Strategy Conclusion		
The Sponsor provided adequate information to support the manufacturing control activities for the essential performance requirements of the combination product.	☒ Yes	☐ No

12.4. Facilities & Quality Systems Review Conclusion

FACILITIES & QUALITY SYSTEMS REVIEW CONCLUSION		
Filing Deficiencies: ☐ Yes ☒ No ☐ N/A	Mid-Cycle Deficiencies: ☒ Yes ☐ No ☐ N/A	Final Deficiencies: ☐ Yes ☒ No ☐ N/A
Reviewer Comments		

The Sponsor provided a complete response to all information requests. The responses were reviewed and found acceptable.

UPDATE JANUARY 11, 2021:

The CDRH portion of the 704a facilities “desk” review was concluded. The provided documents were reviewed and found to provide acceptable evidence that the Rechon facility is able to manufacture the Auto-Injector and Pre-Filled Syringe according to GMP requirements. It is to note that the commercial manufacturing line of the AI is not fully validated yet. Zealand committed to finishing the assembly line validation prior to the PDUFA date. The applicant stated that two modifications were made to the previously validated AI assembly line (b) (4) in order to improve the manufacturing to a reliability content of 99.99%. Hence, **a post-approval inspection is recommended to evaluate the final, commercial AI assembly line validation. Please see Appendix B for the 704a review.**

CDRH sent Facilities & QS Deficiencies or Interactive Review Questions to the Sponsor: Yes No

	Date Sent: 8/11/2020	Date/Sequence Received: 9/18/2020 Seq. 26
Information Request #5	You provided a summary of your CAPA system in 3.2.P.3.3 “21 CFR Part 820 Quality Management system Pre-filled Safety Syringe & Auto-injector”. However, a more detailed description of your CAPA system per 21 CFR 820.100 is needed to ensure issues with the device post-marketing are adequately addressed. Provide a more detailed description of your CAPA system specifically addressing the following: a) Identify the sources of quality data and how this data is analyzed to identify existing and potential causes of nonconforming practices and products. Please specifically describe how these activities are divided between Zealand Pharma, Rechon Life Science, and (b) (4); b) How nonconformities and their causes are investigated; c) A description of how actions needed to correct and prevent recurrence of nonconformities are identified and implemented. This should include a description on how these tasks are divided between Zealand Pharma, Rechon Life Science, and (b) (4); d) A description of the verification or validation activities done in response to a nonconformance; and e) A description of the disposition procedures of nonconforming products.	
Sponsor Response	Response to question 4a Zealand has established procedures for application of corrective and preventive action (CAPA) and ensured that equivalent procedures are in place at facilities involved in the manufacture of the dasiglucagon combination products. The procedures outline the process for identifying, initiating, implementing, verifying, and completing CAPAs. Zealand has overall Quality oversight of its approved products. Zealand has Supplier and Quality Agreements with both the auto-injector and pre-filled safety syringe final assembly site (Rechon Life Science AB, termed Rechon hereafter) and (b) (4) (b) (4) The quality data sources and how they are analyzed to identify existing and potential nonconforming products and practices can be divided into two types; manufacturing quality data and direct output of quality activities.	

Manufacturing quality data are identified during a defined regular activity or process and are managed immediately according to the respective CAPA system of the manufacturer. The CAPA systems of the manufacturing sites are oversighted by Zealand through audits, and according to the respective Quality Agreements any CAPAs impacting Zealand products are to be communicated to Zealand. For CAPAs identified during manufacturing that directly impact Zealand's products a Zealand CAPA is initiated.

(b) (4)

(b) (4)

For quality data sources originating as a direct output of quality activities, e.g. field complaints, returned products, risk assessments, Zealand audits of Rechon and (b) (4) inspections, and overall quality trends, Zealand initiates and handles the impact assessment and investigation. If there are indications that the potential impact or root cause originate from or are connected to the manufacturing quality data described in the tables above (Table 3 and Table 4), (b) (4) Rechon or both parties will be involved in the CAPA handling process. In addition, if the cause might be associated with the design of the auto-injector combination product, (b) (4) will be involved in the investigation and the CAPA process.

Response to question 4b

The nonconformities can be identified through different quality data sources, as described in the Response to question 4a. All nonconforming processes that are directly impacting Zealand products are handled in the Zealand CAPA system. (b) (4)

(b) (4)

The systematic investigation process identifies the appropriate corrections, corrective actions and preventive actions. Any actions identified or linked to the manufacturing quality data described (b) (4) are communicated to the respective CMO. Additionally, if identified actions concern the design of the device-constituent part of the combination product, (b) (4) will handle the specific action as the owner of the auto-injector design. All these actions will be overseen by Zealand and documented in the Zealand Quality System. The action implementation, its effect, and CAPA handling will be subject for future audits of the respective manufacturer.

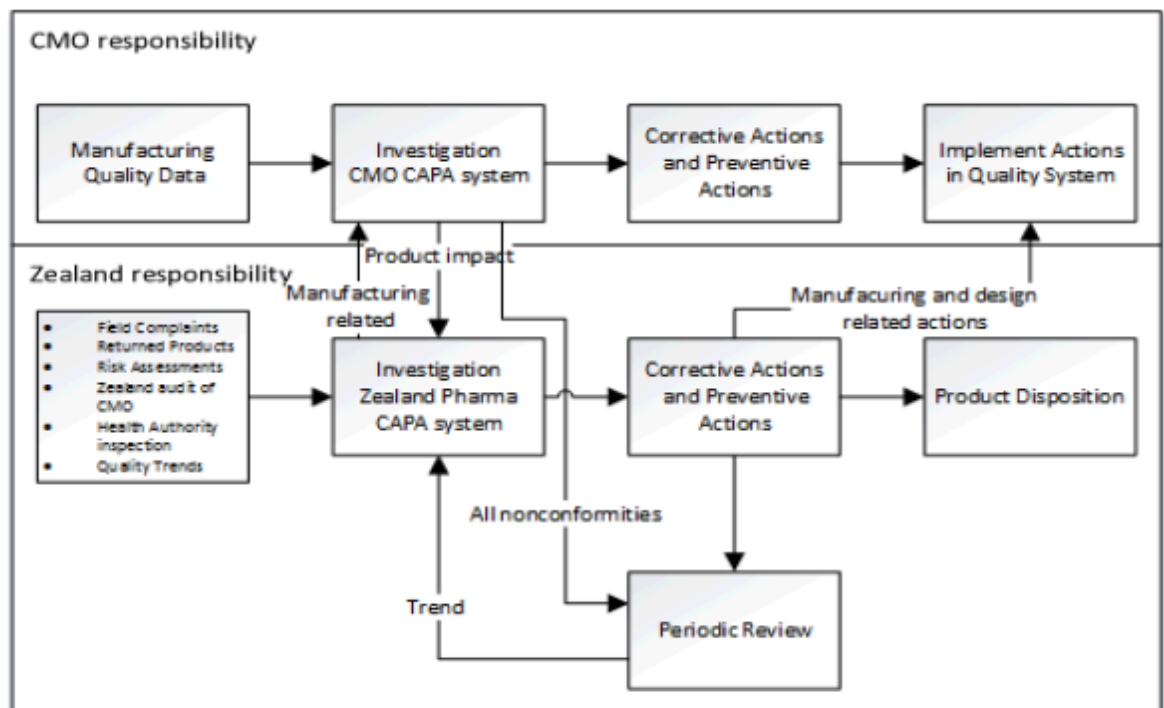


Figure 1 CAPA process and split of responsibility

CAPAs originating from the manufacturing quality data, as identified in [Table 3](#) and [Table 4](#) are handled by the respective CMOs (Rechon or (b) (4)) and are communicated to Zealand, if they have implications on the product.

On a periodic basis Zealand will be to review all nonconformities related to the pre-filled safety syringe and auto-injector with input from Rechon and (b) (4). The periodic review will ensure a proper reaction to nonconforming trends, and to identify if any nonconformities have reoccurred and not identified as reoccurring. An additional investigation will take place to assess reoccurring nonconformities.

	(b) (4)
Reviewer Comments	The Sponsor has provided an overview of the CAPA system including how each activity is divided up between Zealand, (b) (4), and Rechon. The described CAPA system appears adequate for the proposed device and clearly described how quality data is analyzed and used in the CAPA system to decide when corrective actions are needed. The sources of quality data described by Zealand appear reasonable and the way this data is treated is acceptable. Overall, the response the Sponsor provided adequately addresses concerns regarding the CAPA system.
Response Adequate:	☒ Yes ☐ No, See IR # Sent on Click or tap to enter a date.

	Date Sent: 9/3/2020	Date/Sequence Received: 10/2/2020 Seq 28
Information Request #6	In your response to a Question 7 from August 17, 2020 (received in Sequence 0021), you stated that the auto-injector assembly process at Rechon has not been validated. Please note that validation of device assembly procedures for this emergency use product should be carried out prior to approval of the combination product. In addition, it is unclear how devices manufactured at (b) (4) and used for design verification and reliability testing are representative of the commercial product if the intended commercial manufacturing line (Rechon). This information is necessary to ensure that the provided data is representative of the commercial product. In addition, as this is an emergency use device, it is critical that devices are manufactured consistently with a high degree of assurance that the essential performance requirements are met. Provide the following: a) Evidence that the manufacturing lines for auto-injector assembly are validated at the intended commercial manufacturing line prior to release of commercial product b) Clarify whether or not the manufacturing process at (b) (4) is validated c) A rationale of how the test articles used for design verification and reliability testing are representative of the commercial product if the devices were manufactured at (b) (4) d) A rationale of how the assumptions (e.g., effectiveness of manufacturing controls, performance) made in your Fault Tree Analysis (FTA) regarding the reliability of device assembly and release testing are effective if the process has not been validated at Rechon.	

Sponsor Response	Question 1a Evidence that the manufacturing lines for auto-injector assembly are validated at the intended commercial manufacturing line prior to release of commercial product. Response to question 1a Zealand Pharma A/S (termed Zealand hereafter) confirms that the manufacturing line for auto-injector final assembly of commercial products will be validated prior to release of commercial products. (b) (4) Question 1b Clarify whether or not the manufacturing process at (b) (4) is validated. Response to question 1b For the commercial product, (b) (4) is only responsible for manufacture of components and sub-assemblies to be the used in the final assembly of auto-injectors. The final assembly of auto-injectors for commercial use will only be performed by Rechon.

(b) (4) has performed final assembly of auto-injectors used for design verification testing. These auto-injectors were assembled from components and sub-assemblies equivalent to components and sub-assemblies to be used for the commercial product and by a final assembly process equivalent to the commercial final assembly process.

The auto-injector device constituents parts commercial validation plan, including (b) (4) components and sub-assemblies validation, is presented in [Figure 1](#).

Question 1c

A rationale of how the test articles used for design verification and reliability testing are representative of the commercial product if the devices were manufactured at (b) (4).

Response to question 1c

Zealand would like to clarify that final assembly of auto-injectors used in reliability performance testing has been performed at the commercial final assembly manufacturer Rechon employing the same final assembly process and manufacturing line as will be used for commercial auto-injectors. The final assembly process at Rechon has been validated to a probability content level of 99 % for essential performance requirements (EPRs) to support use of the auto-injectors in clinical trials. The final assembly process for commercial manufacture will be validated to a probability content level of 99.99 % for EPRs to meet the requirements for this emergency product, see [Figure 1](#). Hence, the auto-injectors used for reliability performance testing are representative of the commercial auto-injectors.

(b) (4)

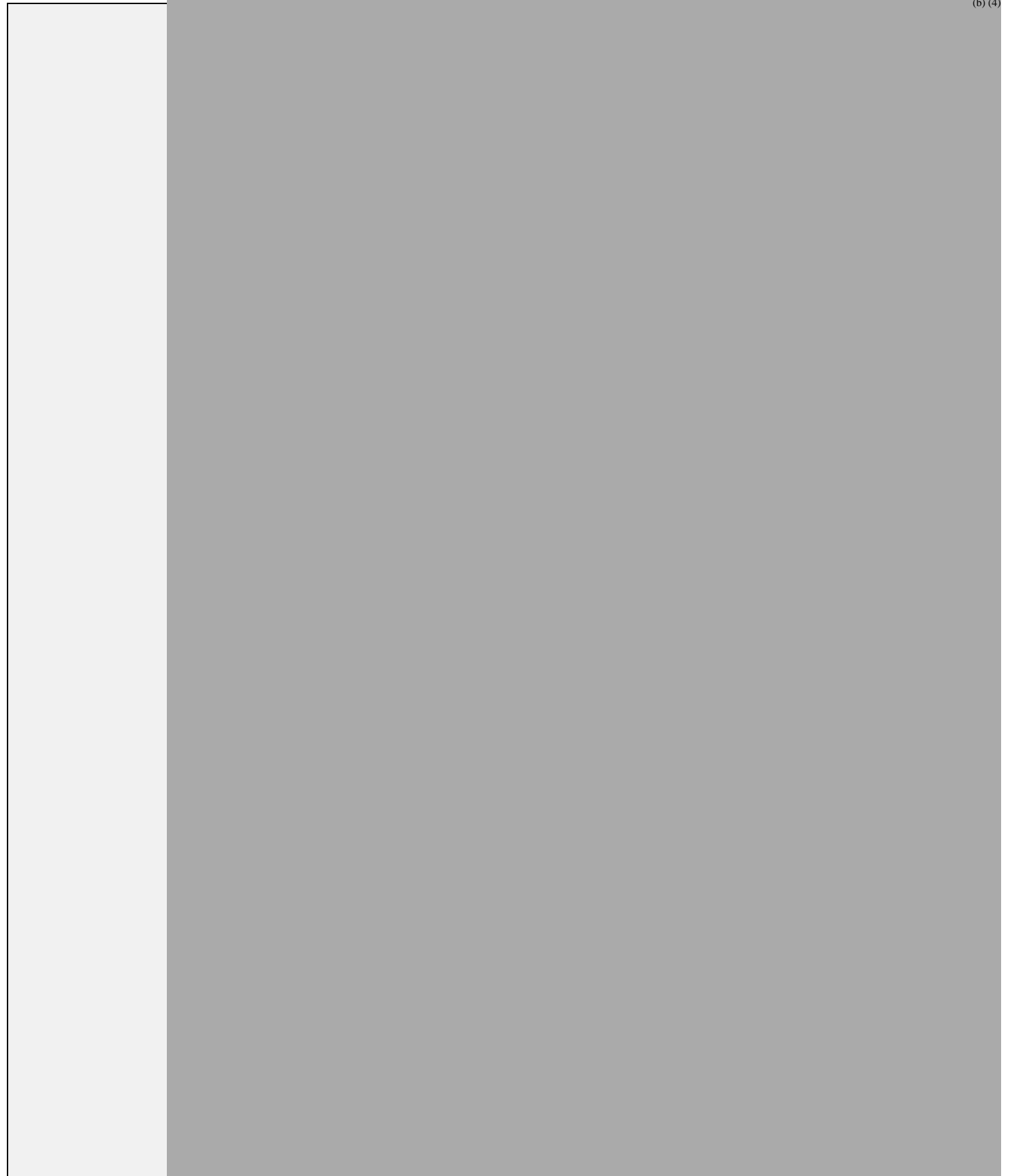
Question 1d

A rationale of how the assumptions (e.g., effectiveness of manufacturing controls, performance) made in your Fault Tree Analysis (FTA) regarding the reliability of device assembly and release testing are effective if the process has not been validated at Rechon.

Response to question 1d

The reliability performance testing performed with dasiglucagon auto-injector batch V0159D1A provides evidence that the manufacturing controls applied during the manufacture of the batch were effective, even if the control level proposed for final assembly of commercial products was not fully implemented at the time of assembly of this batch.

Dasiglucagon auto-injector batch V0159D1A used for reliability testing was manufactured by Rechon using the same manufacturing and final assembly process as will be used for commercial products. Batch V0159D1A originates from the pre-filled syringe process performance qualification batch V0159. For information on the manufacturing process of the pre-filled syringe, see 3.2.P.3.3 Description of Manufacturing Process and Process Controls. There are only minor differences between the (b) (4) controls used during final assembly of batch V0159D1A and the assembly process to be used for commercial manufacturing. (b) (4)



(b) (4)

**Reviewer
Comments**

Question 1a:

Zealand stated that the manufacturing line for commercial batches of the auto-injector will be validated prior to release. A figure was provided (figure 1 above) that includes a timeline of when assembly processes of the AI subassemblies and final AI assembly are planned to be validated. Zealand indicated that validation activities are planned to be completed prior to the PDUFA date, however this timeline is subject to change. Overall, the Sponsor indicated that validation activities are in process, that protocols are in place to prohibit release of product from production lines that have not been validated, and that final release of product is performed by Zealand. This is acceptable as there is no requirement that assembly validation has to be completed prior to adjudication of the file.

Question 1b:

The Sponsor stated that devices used for design verification testing were manufactured and assembled at (b) (4) using *components and sub-assemblies equivalent to components and sub-assemblies to be used for the commercial product by a final assembly process equivalent to the commercial final assembly process*. Based on the Sponsor's response to Question 1a, subassembly validation activities are currently ongoing at (b) (4). The adequacy of this response is dependent on the Sponsor's response to Question 1c.

Question 1c:

Zealand stated that all device subassemblies for design verification (DV) and reliability testing were manufactured and (b) (4) *using an equivalent final assembly process as used at Rechon.* (b) (4)
(b) (4)

	(b) (4)
	Regarding AI assembly, (b) (4) (b) (4) (b) (4) on used the same assembly process and manufacturing line that will be used during commercial manufacturing. This is acceptable. <u>Question 1d:</u> Zealand stated that the assumption made in the fault tree are primarily based on statistical analyses like tolerance intervals and capability analyses. In addition, the Sponsor intends to add additional control to the manufacturing process that were not in place during the manufacturing of test articles for reliability testing. As stated in response to Question 1c, the AIs used during reliability testing were assembled at Rechon using an equivalent assembly process as used during commercial manufacturing which was reviewed and found to support the fault-tree analysis. Hence, this response is acceptable.
Response Adequate:	☒ Yes ☐ No, See IR # Sent on Click or tap to enter a date.

	Date Sent: 9/3/2020	Date/Sequence Received: 10/2/2020 Seq 28
Information Request #7	You are proposing to conduct release testing of the auto-injector and pre-filled syringe at Rechon Life Science AB. However, it is unclear whether these test methods have been validated at this facility. This is necessary to ensure that release testing will be conducted using validated test procedures. Provide evidence that the test procedures used for release testing of the auto-injector and pre-filled syringe have been validated at Rechon Life Science AB.	
Sponsor Response	(b) (4)	



	<i>Conclusion</i> The dedicated injection test equipment used for functional tests of the auto-injector has been appropriately qualified. Furthermore, repeatability and reproducibility of the test equipment was verified to comply with the requirements listed in ISO 11608-1 and ISO 11608-5. Thus, the test equipment is considered suitable for the intended use.
Reviewer Comments	Zealand stated that IQ and OQ were successfully performed at Rechon and provided a summary of the OQ activities. The submitted data indicates that the repeatability and reproducibility are acceptable for the product (e.g., Gauge R&R values of the test results range from (b) (4). Based on the prior responses, it appears that PQ is still in progress at Rechon. <u>Additional information regarding these procedures was requested during the CDER 704a and will be reviewed under this request for information.</u> The response to this IR is acceptable.

Response Adequate:	☒ Yes ☐ No, See IR # Sent on Click or tap to enter a date.

<<END OF REVIEW>>

13. APPENDIX A (INFORMATION REQUESTS)

13.1. Filing/74-Day Information Requests

1. You provided a summary of your device verification activities for the Pre-Filled Syringe and Auto-Injector. However, you have not provided full test protocols including a description of the test methods and data. This information is necessary for the review of the submission. Provide full test reports for the conducted verification activities of the device constituent parts of the combination product. Test reports should include a description of the test method, a description of the test article, and test results.

13.2. Mid-Cycle Information Requests

2. You stated that you evaluate cap removal force as an essential performance requirement of your proposed auto-injector with an acceptance criterion is $\leq \frac{(b)}{(4)} N$. This specification appears high and your actual test results for cap removal force are much lower (mean test results after preconditioning were between $\frac{(b)}{(4)} N$ according to 3.2.R Device - Design Verification Summary - Auto-injector). Due to the large difference between test results and your acceptance criterion, it is unclear how the conducted clinical or human factors study validates the cap removal specification. Provide additional validation data (e.g., literature review) to support your cap removal specification or tighten your specification to be closer to the measured forces.
3. In 3.2.R “Device – Design Verification Summary – Pre-filled Safety Syringe” and 3.2.R “Device – Design Verification Summary – Auto-injector”, you stated that accelerated aging was done for 52 days at 45°C with subsequent aging for 6 months at 25°C for device performance testing. However, it is unclear how these aging parameters are representative of your proposed shelf-life of 3 years when stored at $\frac{(b)}{(4)} C$ with the option to store the product for 1 year at 25°C. Provide a rationale of how your accelerated aging with subsequent 6 months storage at 25°C supports your shelf-life claim or conduct additional testing of devices that underwent accelerated and/or real-time aging representative of $\frac{(b)}{(4)}$.
4. You provided results of Needle trap activation force and deactivation force testing for the pre-filled safety syringe (PFSS) in Seq 008 3.2.R “Device – Design Verification Plan Report – Pre-filled Safety Syringe”. You stated these performance attributes were verified with 30 samples each to a $\frac{(b)}{(4)}\%$ probability content with $\frac{(b)}{(4)}\%$ confidence. However, per the 2005 FDA guidance *Medical Devices with Sharps Injury Prevention Features* (<https://www.fda.gov/media/71142/download>), sharps injury prevention features should be verified to a 95%/99% confidence/reliability. Provide additional testing of the sharps prevention feature of the PFSS to demonstrate that the device meets these performance attributes with 95% confidence and 99% reliability.
5. You provided a summary of your CAPA system in 3.2.P.3.3 “21 CFR Part 820 Quality Management system Pre-filled Safety Syringe & Auto-injector”. However, a more detailed description of your CAPA system per 21 CFR 820.100 is needed to ensure issues with the device post-marketing are adequately addressed. Provide a more detailed description of your CAPA system specifically addressing the following:
 - a. Identify the sources of quality data and how this data is analyzed to identify existing and potential causes of nonconforming practices and products. Please specifically describe how these activities are divided between Zealand Pharma, Rechon Life Science, and $\frac{(b)}{(4)}$
 - b. How nonconformities and their causes are investigated;
 - c. A description of how actions needed to correct and prevent recurrence of nonconformities are identified and implemented. This should include a description on how these tasks are divided between Zealand Pharma, Rechon Life Science, and $\frac{(b)}{(4)}$;
 - d. A description of the verification or validation activities done in response to a nonconformance; and
 - e. A description of the disposition procedures of nonconforming products.

13.3. Interactive Information Requests

13.3.1. Interactive Information Requests sent on 9/3/2020

6. In your response to a Question 7 from August 17, 2020 (received in Sequence 0021), you stated that the auto-injector assembly process at Rechon has not been validated. Please note that validation of device assembly procedures for this emergency use product should be carried out prior to approval of the combination product. In addition, it is unclear how devices manufactured at (b) (4) and used for design verification and reliability testing are representative of the commercial product if the intended commercial manufacturing line (Rechon). This information is necessary to ensure that the provided data is representative of the commercial product. In addition, as this is an emergency use device, it is critical that devices are manufactured consistently with a high degree of assurance that the essential performance requirements are met. Provide the following:
 - a) Evidence that the manufacturing lines for auto-injector assembly are validated at the intended commercial manufacturing line prior to release of commercial product
 - b) Clarify whether or not the manufacturing process at (b) (4) is validated
 - c) A rationale of how the test articles used for design verification and reliability testing are representative of the commercial product if the devices were manufactured at (b) (4)
 - d) A rationale of how the assumptions (e.g., effectiveness of manufacturing controls, performance) made in your Fault Tree Analysis (FTA) regarding the reliability of device assembly and release testing are effective if the process has not been validated at Rechon.
7. You are proposing to conduct release testing of the auto-injector and pre-filled syringe at Rechon Life Science AB. However, it is unclear whether these test methods have been validated at this facility. This is necessary to ensure that release testing will be conducted using validated test procedures. Provide evidence that the test procedures used for release testing of the auto-injector and pre-filled syringe have been validated at Rechon Life Science AB.

13.3.2. Interactive Information Requests sent on 10/9/2020

8. In your response to Question 2 of our information request dated August 27, 2020, you stated that, when calculating your accelerated aging time, you used an aging factor (b) (4) instead of the commonly utilized Q_{10} of 2.0. Per ASTM F1980-16 *Standard Guide for Accelerated Aging of Sterile Barrier Systems for Medical Devices*, a more aggressive reaction rate coefficient may only be chosen if the system under investigation is sufficiently characterized in the literature. We are not aware of available information that would substantiate use of a more aggressive aging factor. Re-calculate the shelf-life of the device constituent parts of your combination product using a Q_{10} factor of 2.0. Alternatively, provide a scientific justification and literature data that would support the use of an aging factor (b) (4) for your device.

13.3.3. Interactive Information Requests sent on 11/10/2020

9. In your response to our information request dated October 13, 2020, you stated that your accelerated aging parameters for the auto-injector are based on activation energies of degradation reactions (e.g., oxidation, hydrolysis, etc.) of some materials used in the construction of the device. However, this assessment does not cover all materials or components used in the manufacture of the device and does not consider how the system is projected to age as a whole. Per ASTM F1980-16, the *system under investigation* should be characterized in the literature to justify less conservative accelerated aging parameters. Furthermore, as your device is intended for emergency use, conservative preconditioning parameters should be chosen to ensure that your device performs

adequately throughout its shelf-life. We disagree that your aging protocol provides a conservative means of ensuring that your device performs adequately throughout the intended shelf-life. Provide ONE of the following:

- a. Recalculate your shelf-life using a Q_{10} factor of 2.0; OR
- b. Provide additional testing of devices that underwent accelerated aging (using a Q_{10} factor of 2.0) representative of the device stored for [REDACTED]^{(b)(4)}; OR
- c. Provide performance data from real-time aged devices stored for [REDACTED]^{(b)(4)} at 25°C to support your shelf-life claim.

14.APPENDIX B (704a Facilities “Desk” Review)

#	Request	Response or Attachment Reference	Assessment	Adequate (Y/N)
1	Provide a summary of how your firm’s management has established responsibility to assure that the combination product is manufactured in compliance with all applicable CGMP requirements. In addition to the summary please provide the following: a) Provide a description of the functions and responsibilities of each facility involved in the manufacturing of the combination product and its constituent parts. b) Also, provide procedures in support of your overview summary which govern the processes for assuring that the combination product is manufactured in compliance with all cGMP requirements.	<i>The response to #1 is provided both by Rechon Life Science AB (Rechon) and Zealand Pharma A/S (Zealand). The reasoning is stated in Preamble to the FDA inspection Records Request</i> <i>Rechon response reference:</i> • <i>DR 1 Rechon responsibilities and QMS</i> <i>Rechon response attachment:</i> • <i>SOP 690-1000 Rechon Quality Manual</i> <i>Zealand response reference:</i> • <i>DR 1 Zealand – Management responsibility</i> <i>Zealand response attachment:</i> • <i>SOP-0001 Quality Manual</i> • <i>SOP-0084 Monitoring of external compliance requirements</i> • <i>SOP-0101 Training</i> • <i>SOP-0209 Internal Audits</i>	Provided for Rechon. Management review meetings are held on quarterly basis discussing quality related issues (e.g., CAPAs, deviations, environmental control, supplier management, etc.). Participation of management team is mandatory (CEO, Head of P&V, and Head of QA). The head of QA is identified as the person responsible to lead management review meetings.	Y

		<i>SOP-0217 Quality Management Review</i>		
2	Explain how your firm has utilized the design control process to develop the combination product under review. In addition to the summary please provide the following: a) Provide an overview of your design control program and provide associated procedures which govern the design control program. b) Please address how requirements for design and development planning, design input, design output, design review, design verification, design validation, design transfer, design changes, and design history file are being satisfied. c) Provide a copy or a summary of the plan used to design the combination product.	<i>The response to #2 is provided by Zealand. The reasoning is stated in Preamble to the FDA inspection Records Request</i> <i>Zealand response reference:</i> <i>DR 2 Zealand – Design Control</i> <i>Zealand response attachment:</i> <i>SOP-0181 Device and Packaging Development</i> <i>SOP-0220 Medical Device Risk Management</i> <i>SOP-0222 Change Control</i> <i>Design and Development Plan ZP4207SD AI</i> <i>Design and Development Plan ZP4207SD PFSS</i>	Stated that design control activities are managed by Zealand. SOP-0222 describes how product changes proposed by CMOs are handled by Zealand. (b) (4) (b) (4)	Y
3	Provide an overview of the procedure(s) for purchasing controls. Provide procedures which describe the following: a) Describe your supplier evaluation process and describe how it will determine the type and extent of control you will exercise over suppliers.	<i>The response to #3 is provided both by Rechon and Zealand. The reasoning is stated in Preamble to the FDA inspection Records Request</i>	Rechon provided an overview of the purchasing controls as part of their QMS. (b) (4)	Y

	b) Explain how you maintain records of acceptable suppliers and how you address the purchasing data approval process. c) Explain how you will balance purchasing assessment and receiving acceptance to ensure that products and services are acceptable for their intended use. d) Explain how that changes made by contractors/suppliers will not affect the final combination product. e) Provide a description of how you apply the purchasing controls to the suppliers/contractors used in the manufacturing of the combination product (e.g., through supplier agreements).	<i>Rechon response reference:</i> • <i>DR 3 Rechon Purchasing Controls</i> <i>Rechon response attachment:</i> • <i>xxx</i> <i>Zealand response reference:</i> • <i>DR 3 Zealand – Purchasing Controls</i> <i>Zealand response attachment:</i> • <i>SOP-0077 Vendor Management</i> • <i>SOP-0169 CMC Vendor Selection & Oversight</i> • <i>SOP-0190 Handling of change requests and deviations from CMOs</i> • <i>SOP-0194 Quality Agreements</i> • <i>SOP-0210 External Audits</i> • <i>SOP-0222 Change Control</i>	(b) (4)	
5	Provide the procedure(s) for your corrective and preventive action (CAPA) system. The CAPA system procedures provided should include: a) Identification of sources of quality data and analysis of these data to identify existing and potential causes of nonconforming practices and products; b) Investigation of nonconformities and their causes;	<i>The response to #5 is provided both by Rechon and Zealand. The reasoning is stated in Preamble to the FDA inspection Records Request</i> <i>Rechon response reference:</i>	The quality agreement between Zealand and Rechon states that Rechon is responsible for investigating root causes and implementation of CAPAs for issues related the manufacturing quality data. The provided information includes SOP 690-3800 describing the CAPA procedures in place at Rechon. (b) (4)	Y

	c) Identification and implementation of actions needed to correct and prevent recurrence of nonconformities; and d) Verification or validation of the actions taken.	• <i>DR 5 Information on the Rechon CAPA system</i> <i>Rechon response attachment:</i> • <i>SOP 690-3900-04 Translation</i> • <i>SOP 690-3900-04 & att 1-7 CAPA system</i> <i>Zealand response reference:</i> • <i>DR 5 Zealand – CAPA system</i> <i>Zealand response attachment:</i> • <i>SOP-0068 Deviation and CAPA handling</i> • <i>SOP-0190 Handling of change requests and deviations from CMOs</i> • <i>SOP-0194 Quality Agreements</i> • <i>SOP-0210 External Audits</i> • <i>SOP-0217 Quality Management Review</i>	(b) (4)	
13	Provide the equipment installation, operation and performance qualification protocols and reports for the syringe filling machine, auto-injector assembly equipment and pre-filled syringe assembly equipment.	<i>The qualification documents according to list in DR 13 are provided in three folders:</i> <i>Qualification of Syringe filling line</i>	Received – The operational qualification of the AI assembly machine is not complete (b) (4)	N (see below 13.1)

		<i>Qualification of autoinjector assembly machine 03-FAM03</i> <i>Qualification of PFSS assembly equipment</i>	(b) (4)	
13.1	In response to 704(a) Document Request item #13 you have provided qualifications for the syringe filling machine, auto-injector assembly equipment and pre-filled syringe assembly equipment. You have indicated the Operational Qualification of the AI equipment requires completion however we could not locate rationale for reperformance of the Performance Qualification of the AI equipment. Please indicate how performance qualifications for the AI equipment will be addressed.	<i>The following documents have been provided:</i> - <i>Information on PQ of the autoinjector assembly equipment</i> - <i>PQ Protocol QGE-4033-01</i> - <i>Updated list of equipment qualification status ref.</i> - <i>Doc.Req.#12</i>	Received - (b) (4) (b) (4) (b) (4). Sponsor stated that final validation of commercial line will be completed prior to NDA PDUFA date. Information provided by Zealand in Seq 28 of NDA. As this is a high risk device (Emergency use injector), recommend post-approval inspection to assess final commercial AI assembly.	Y

14	Provide the manufacturing process validation protocol (PPQ) along with any written procedures and study reports related to process validation activities, if available for Dasiglucagon Injection finished product manufacture, which includes all manufacturing unit operations such as Drug Product Compounding and Filling, AI Assembly and Packaging and PFS Assembly and Packaging.	<i>DR 14 Summary of Information</i> <i>QGS-370121-00 Protocol PPQ PFS</i> <i>Due to size issues the PPQ report was divided in two:</i> <i>QGT-370121-01 Report PPQ PFS page 1 to 20</i> <i>QGT-370121-01 Report PPQ</i> <i>SOP 690-3605-03 & Att 1-4 Process Validation</i>	Received – (b) (4) (b) (4) The applicant has committed to completing process validation prior to finish drug product distribution for commercialization. (b) (4)	Y
15	Provide prefilled syringe and auto-injector functional parameter development studies and associated data.	<i>The response to #15 is provided by Zealand. The reasoning is stated in Preamble to the FDA inspection Records Request</i> <i>Zealand response reference:</i> <i>DR 15 Zealand – Development of functional paramters</i>	Design Control/product development activities are managed by Zealand. The provided documents were reviewed as part of the device engineering review.	Y

		<i>Zealand response attachment:</i> • <i>3.2.R Device - Design Verification Summary - Auto-injector</i> • <i>3.2.R Device - Design Verification Summary - Pre-filled Safety Syringe</i> • <i>IDA0085 Design Verification - Feature - Protocol and Report</i> • <i>IDA0085 Design Verification - Performance - Protocol and Report</i> • <i>IDA0085 Design Verification - Reliability - Protocol and Report</i> • <i>IDA0085 Design Verification Plan Report</i> • <i>Transport Test Report and Protocol ZP4207SD AI</i> • <i>Transport Test Report ZP4207SD PFSS</i>		
23	Please provide documentation (written test methods and study results) of the validation of release test methods for the pre-filled syringe and auto-injector functional performance tests (e.g., dose accuracy, activation force, etc.).	<i>DR 23 AI_PFSS Functional Release Tests</i> <i>Qualification documents:</i> <i>QGB-4037-00 Add 5-00</i> <i>Qualification (b) (4)</i> <i>(b) (4)</i> <i>QGR-8391-00 Qualification (b) (4)</i>	(b) (4) The qualification is acceptable. Follow up required. (b) (4) (b) (4)	Y (see 23.1 for further follow-up below)

		<i>Methods:</i> <i>QD-038-03 Test method plunger force</i> <i>QE-464-04 Test method AI functional tests</i>	Request PQ protocol and report (b) (4) (b) (4)	
23.1	In request #23, you have submitted “QGR-8391-00 Qualification (b) (4) Please provide reference [8] “QGE-8391-00” and [9] “QGG-8391-00”.	<i>The requested qualification documents are provided:</i> <i>QGE-8391-00 (b) (4) PQ-protocol</i> <i>QGG-8391-00 (b) (4) PQ-report</i>	Qualification (b) (4) was reviewed and found acceptable.	Y

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

HAMET M TOURE
02/03/2021 10:31:47 AM

MEMORANDUM
REVIEW OF REVISED LABEL AND LABELING
Division of Medication Error Prevention and Analysis (DMEPA)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

Date of This Memorandum: January 21, 2021
Requesting Office or Division: Division of Diabetes, Lipid Disorders, and Obesity (DDLO)
Application Type and Number: NDA 214231
Product Name and Strength: Zegalogue (dasiglucagon) injection, 0.6 mg/0.6 mL
Applicant/Sponsor Name: Zealand Pharma A/S
OSE RCM #: 2020-614-1
DMEPA Safety Evaluator: Colleen Little, PharmD
DMEPA Team Leader: Lolita White, PharmD

1 PURPOSE OF MEMORANDUM

The Applicant submitted revised Instructions for Use (IFU), (b) (4) container labels, and carton labeling received on December 11, 2020 for Zegalogue. Division of Diabetes, Lipid Disorders, and Obesity (DDLO) requested that we review the revised IFUs, (b) (4) container labels, and carton labeling for Zegalogue (Appendix A) to determine if they are acceptable from a medication error perspective. The revisions are in response to recommendations that we made during a previous Human Factors study report and label and labeling review.^a

2 CONCLUSION

The revised IFUs, prefilled syringe (PFS) (b) (4), and autoinjector (AI) container label and carton labeling are unacceptable from a medication error perspective. The labels and labeling do not include important information for dose administration and the AI carton labeling includes a device descriptor which may lead to confusion. Additionally, we reiterate our previous recommendation to revise the instruction and image on the protective case. We provide recommendations to address these concerns in Section 3 below.

^a Little, C. Human Factors Study Report and Label and Labeling Review for Zegalogue (NDA 214231). Silver Spring (MD): FDA, CDER, OSE, DMEPA (US); 2020 DEC 10. RCM No.: 2020-614 and 2020-724.

3 RECOMMENDATIONS FOR ZEALAND PHARMA A/S

We recommend the following be implemented prior to approval of this NDA:

A. Autoinjector (AI) carton labeling

1. As presented, the location of the device description (b) (4) may contribute to confusion (b) (4)

(b) (4)

(b) (4). Revise the AI carton labeling to either remove or relocate the term (b) (4) so that it does not immediately follow the proprietary name.

B. AI container label

1. The “back” image under the “Choose site Expose Skin” step is inconsistent with the injection sites identified in the prescribing information (PI). We are concerned that inconsistent information regarding the appropriate injection site can cause confusion, which may lead to wrong technique medication error. Revise the “back” image under the “Choose site Expose Skin” step to include the back of thighs injection sites.

C. Prefilled syringe (PFS) (b) (4) protective case labeling

1. The critical instruction to expose bare skin prior to administering the injection is not presented (b) (4). We acknowledge your response to our November 30, 2020 information request^b, however we disagree with your assertion that selection of the injection site is a more important instruction to emphasize compared to exposing the bare skin. According to your use related risk analysis, failure to expose skin may result in a dose omission medication error. Thus, the failure to complete the task to expose bare skin may result in patient harm. As such, this critical instruction should be presented consistently throughout all labels and labeling. Additionally, we remind you that two participants who failed this task provided subjective feedback in your Human Factors (HF) validation studies to indicate that your (b) (4) and AI container label could be improved to include instructions to expose skin prior to administration. Revise the statement, (b) (4) to “Choose site Expose skin” to align with the IFU and AI (b) (4). We acknowledge your concern regarding space limitations. As such, consider decreasing the font size of the words “prefilled syringe” to provide additional space for this revision.

2. We note that you have not implemented our recommendation to revise the instruction and image related to the open the protective case task to appear

^b Responses to FDA Information Request dated 30 Nov 2020 to Carton and Container Labels for dasiglucagon NDA 214231. Soborg, Denmark: Zealand Pharma A/S; 2020 DEC 11. Available from:

<\\CDSESUB1\evsprod\nda214231\0039\m1\us\comments-req-resp-ir-30nov2020-carton-container-labels.pdf>

vertically. We acknowledge your response to our November 30, 2020 information request; however, we disagree that the proposed image “has been optimized.” You state that the use errors related to opening the protective case in the HF validation study for the PFS are “attributed to first time discovery and would not be expected in subsequent use.” However, we disagree because your proposed product is an emergency use product. Thus, failures attributed to “first time discovery” could cause serious harm including compromised medical care (i.e., severe hypoglycemia). We also refer to your HF validation study results for the task to open the protective case where the root cause analysis attributes 2 use errors to information oversight which could be related to the orientation of the instruction and image. As such, we are still concerned that as proposed, the presentation of this step on the (b) (4) for the PFS may lead to confusion leading to patient harm. We recommend that you consider decreasing the size of the barcode on the PFS (b) (4) and consider revising the instruction and image related to the open the protective case task to appear vertically when the protective is in a horizontal position. If you decrease the size of the barcode, please ensure that the shrunken barcode is readable/scannable.

D. AI and PFS IFUs

1. The “After injection” section is inconsistent with the PI because it does not inform users that an additional dose may be administered after 15 minutes. We are concerned that inconsistent dosing information can cause confusion, which may lead to a delay in therapy. Revise the “After injection” section of the AI and PFS IFUs to include a statement similar to what is found in the PI which states, “If there has been no response after 15 minutes, an additional dose of ZEGALOGUE may be administered while waiting for emergency assistance.”

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

COLLEEN L LITTLE
01/21/2021 07:40:36 PM

LOLITA G WHITE
01/25/2021 10:56:11 AM

**Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Medical Policy**

PATIENT LABELING REVIEW

Date: January 21, 2021

To: Liz Godwin
Regulatory Project Manager
**Division of Diabetes, Lipid Disorders, and Obesity
(DDLO)**

Through: LaShawn Griffiths, MSHS-PH, BSN, RN
Associate Director for Patient Labeling
Division of Medical Policy Programs (DMPP)

Marcia Williams, PhD
Team Leader, Patient Labeling
Division of Medical Policy Programs (DMPP)

From: Sharon W. Williams, MSN, BSN, RN
Senior Patient Labeling Reviewer
Division of Medical Policy Programs (DMPP)

Samantha Bryant, PharmD, BCPS
Regulatory Review Officer
Office of Prescription Drug Promotion (OPDP)

Subject: Review of Patient Labeling: Patient Package Insert (PPI) and
Instructions for Use (IFU)

Drug Name (established name): ZEGALOGUE (dasiglucagon)

Dosage Form and Route: injection, for subcutaneous use
Application
Type/Number: NDA 214231

Applicant: Zealand Pharma A/S

1 INTRODUCTION

On March 27, 2020, Zealand Pharma A/S., submitted for the Agency's review a New Drug Application (NDA) for dasiglucagon injection, for subcutaneous use. The proposed proprietary name, ZEGALOGUE was submitted to the FDA on November 25, 2019 and approved. ZEGALOGUE is intended for the treatment of severe hypoglycemia in patients with diabetes ages six years and above.

This collaborative review is written by the Division of Medical Policy Programs (DMPP) and the Office of Prescription Drug Promotion (OPDP) in response to a request by the Division of Diabetes, Lipid Disorders, and Obesity (DDLO) on March 31, 2020 for DMPP and OPDP to review the Applicant's proposed PPI and IFUs for ZEGALOGUE (dasiglucagon) injection, for subcutaneous use.

2 MATERIAL REVIEWED

- Draft ZEGALOGUE (dasiglucagon) PPI and IFUs received on March 27, 2020, and received by DMPP and OPDP on January 8, 2021.
- Draft **ZEGALOGUE (DASIGLUCAGON)** Prescribing Information (PI) received on March 27, 2020, revised by the Review Division throughout the review cycle, and received by DMPP and OPDP on January 8, 2021.

3 REVIEW METHODS

In 2008 the American Society of Consultant Pharmacists Foundation (ASCP) in collaboration with the American Foundation for the Blind (AFB) published *Guidelines for Prescription Labeling and Consumer Medication Information for People with Vision Loss*. The ASCP and AFB recommended using fonts such as Verdana, Arial or APHont to make medical information more accessible for patients with vision loss.

In our collaborative review of the PPI and IFUs we:

- simplified wording and clarified concepts where possible
- ensured that the PPI and IFUs were consistent with the Prescribing Information (PI)
- ensured that PPI and IFUs were free of promotional language or suggested revisions to ensure that it is free of promotional language
- ensured that the PPI and IFUs meet the criteria as specified in FDA's Guidance for Useful Written Consumer Medication Information (published July 2006)

4 CONCLUSIONS

- The PPI and IFUs are acceptable with our recommended changes.

5 RECOMMENDATIONS

- Please send these comments to the Applicant and copy DMPP and OPDP on the correspondence.
- Our collaborative review of the PPI and IFUs are appended to this memorandum. Consult DMPP and OPDP regarding any additional revisions made to the PI to determine if corresponding revisions need to be made to the PPI and IFUs.

Please let us know if you have any questions.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

SHARON W WILLIAMS
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MARCIA B WILLIAMS
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SAMANTHA E BRYANT
01/21/2021 10:47:55 AM

**FOOD AND DRUG ADMINISTRATION
Center for Drug Evaluation and Research
Office of Prescription Drug Promotion**

*****Pre-decisional Agency Information*****

Memorandum

Date: January 15, 2021

To: Elizabeth Godwin, Regulatory Project Manager
Division of Diabetes, Lipid Disorders, and Obesity (DDLO)

Monika Houstoun, Associate Director for Labeling, (DDLO)

From: Samantha Bryant, Regulatory Review Officer
Office of Prescription Drug Promotion (OPDP)

CC: Melinda McLawhorn, Team Leader, OPDP

Subject: OPDP Labeling Comments for ZEGALOGUE (dasiglucagon) injection, for subcutaneous use

NDA: 214231

In response to DDLO consult request dated March 31, 2020, OPDP has reviewed the proposed product labeling (PI), patient package insert (PPI), Instructions for Use (IFU), and carton and container labeling for the original NDA submission for ZEGALOGUE.

Labeling: OPDP's comments on the proposed labeling are based on the draft labeling received by electronic mail from DDLO (Liz Godwin) on January 8, 2021, and are provided below.

A combined OPDP and Division of Medical Policy Programs (DMPP) review will be completed, and comments on the proposed PPI and IFU will be sent under separate cover.

Carton and Container Labeling: OPDP has reviewed the attached proposed carton and container labeling received by electronic mail from DDLO (Liz Godwin) on January 11, 2021, and we do not have any comments.

Thank you for your consult. If you have any questions, please contact Samantha Bryant at (301) 348-1711 or Samantha.Bryant@fda.hhs.gov.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

SAMANTHA E BRYANT
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HUMAN FACTORS STUDY REPORT AND LABELS AND LABELING REVIEW
Division of Medication Error Prevention and Analysis (DMEPA)
Office of Medication Error Prevention and Risk Management (OMEPRM)
Office of Surveillance and Epidemiology (OSE)
Center for Drug Evaluation and Research (CDER)

*** This document contains proprietary information that cannot be released to the public***

Date of This Review:	December 10, 2020
Requesting Office or Division:	Division of Diabetes, Lipid Disorders, and Obesity (DDLO)
Application Type and Number:	NDA 214231
Product Type:	Combination Product (Drug-Device)
Drug Constituent Name and Strength	Zegalogue (dasiglucagon) injection, 0.6 mg/0.6 mL
Device Constituent:	Autoinjector and Prefilled Syringe
Rx or OTC:	Rx
Applicant/Sponsor Name:	Zealand Pharma A/S (Zealand)
Submission Date:	March 27, 2020, May 12, 2020, June 16, 2020, June 17, 2020, June 23, 2020, August 10, 2020, September 30, 2020, December 2, 2020
OSE RCM #:	2020-614 and 2020-724
DMEPA Safety Evaluator:	Colleen Little, PharmD
DMEPA Team Leader:	Lolita White, PharmD
DMEPA Associate Director for Human Factors (Acting):	Jason Flint, MBA, PMP
DMEPA Associate Director for Nomenclature and Labeling:	Mishale Mistry, PharmD, MPH

1. REASON FOR REVIEW

This review evaluates the human factors (HF) validation study report and labels and labeling submitted under NDA 214231 for Zegalogue (dasiglucagon) injection.

1.1 PRODUCT DESCRIPTION

Zegalogue (dasiglucagon) injection is supplied in two presentations: autoinjector (AI) and prefilled syringe (PFS). These are combination products that are intended to treat severe hypoglycemia in patients with diabetes aged 6 years and above. See Appendix A.

1.2 REGULATORY HISTORY RELATED TO THE PROPOSED PRODUCT'S HUMAN FACTORS DEVELOPMENT PROGRAM

We provided preliminary comments for the June 29, 2017 End of Phase 2 Type B meeting under IND 127866 in which we acknowledged Zealand's plan to conduct and submit a use-related risk analysis and a Human Factors (HF) validation study protocol for Agency review prior to performing the study.¹

On April 17, 2019, Zealand submitted their HF validation study protocols under IND 127866. Our review of the HF validation study protocols identified several areas of concern. We communicated our recommendations to Zealand in a HF Validation Study Protocol Advice Letter.²

On July 2019, Zealand submitted clarifying questions that were in response to the recommendations in the HF Validation Study Protocol Letter. Specifically,

- Zealand requested Agency feedback for their approach for the untrained user groups.
- Zealand maintained their proposal to include adolescent participants 12 years to 17 years of age and requested reconsideration for our recommendation to include participants as young as 10 years of age.

¹ White, M. Preliminary Meeting Comments for IND 127866. Silver Spring (MD): FDA, CDER, ODEII, DMEP (US); 23 JUN 2017. Available from: https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af80446178&_afRedirect=403487347449682

² Vora, N. Human Factors Validation Study Advice for IND 127866. (MD): FDA, CDER, DMEPA (US); 14 JUN 2019. Available from: https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af804fd415&_afRedirect=404991071934740

We agreed with Zealand's plan to only include untrained user groups in the HF validation study and we recommended that the study include a single visit instead of the proposed separate self-familiarization visit.

We consulted the Division of Pediatrics and Maternal Health (DPMH) to inform our evaluation of Zealand's proposal to include adolescent participants 12 years to 17 years of age. DPMH recommended inclusion of children as young as 8 years in the proposed HF studies. However, after further internal discussion, we decided to maintain our previous recommendation for Zealand to include participants as young as 10 years of age and we asked Zealand to submit additional data to support a different age range other than our previously recommended age range.³

On March 27, 2020, Zealand submitted the results of the HF validation studies for the prefilled syringe and autoinjector presentations under NDA 214231, which are the subject of this review.



³ White, L. Memorandum Review of Human Factors Clarifying Questions for IND 127866. Silver Spring (MD): FDA, CDER, OSE, DMEPA (US); 2019 OCT 10. RCM No.:2019-861-1.





On August 26, 2020, we sent a General Advice Letter to inform Zealand that their (b) (4) proposed PFS presentation was found to be acceptable.⁵ During the September 14, 2020 Mid-Cycle Meeting, Zealand stated their plans to submit an amendment (b) (4) (b) (4).⁶ Subsequently, on September 30, 2020, Zealand submitted an Amendment under NDA 214231 which included revised labels and labeling and Instructions for Use (IFU) for the PFS presentation (b) (4).

2. MATERIALS REVIEWED

We considered the materials listed in Table 1 for this review. The Appendices provide our findings and evaluation of each material reviewed.

Table 1. Materials Considered for this Review	
Material Reviewed	Appendix Section (for Methods and Results)
Product Information/Prescribing Information	A
Background Information Previous HF Reviews (DMEPA and CDRH)	B
Background Information on Human Factors Engineering (HFE) Process	C
Human Factors Validation Study Report	D

⁵ Godwin, E. General Advice Letter for dasiglucagon (NDA 214231). Silver Spring (MD): FDA, CDER, OCHEN, DDLO (US); 2020 AUG 26. Available from: https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af8058edc6&_afRedirect=1507224045952372

⁶ Godwin, E. NME Mid-Cycle Communication for dasiglucagon (NDA 214231). Silver Spring (MD): FDA, CDER, OCHEN, DDLO (US); 2020 SEP 29. Available from: https://darrts.fda.gov/darrts/faces/ViewDocument?documentId=090140af8059b8a6&_afRedirect=1508312422358329

Table 1. Materials Considered for this Review	
Material Reviewed	Appendix Section (for Methods and Results)
Information Requests Issued During the Review	E
Labels and Labeling	F

3. OVERALL ASSESSMENT OF MATERIALS REVIEWED

The sections below provide a summary of the study design, errors/close calls/use difficulties observed, and our analysis to determine if the results support the safe and effective use of the proposed product.

3.1 SUMMARY OF STUDY DESIGN

Table 2 presents a summary of the HF validation study design.

Table 2. Study Methodology for Human Factors (HF) Validation Study	
Study Design Elements	Details
Participants	<u>Autoinjector Study</u> • 15 First Responders (FR) • 15 Injection Experienced Lay Caregivers (CG-EX) • 15 Injection Naive Lay Caregivers (CG-IN) • 16 Adolescent Lay Caregivers (CG-AD) ○ Age range: 10-17 years old <u>Prefilled Syringe Study</u> • 15 First Responders (FR) • 15 Injection Experienced Lay Caregivers (CG-EX) • 15 Injection Naive Lay Caregivers (CG-IN) • 15 Adolescent Lay Caregivers (CG-AD) ○ Age range: 11-17 years old
Training	Formal training was not provided on the use of the AI or PFS; however, if during the introductory conversation with study staff participants indicated that in a real-world setting they would refer to the instructions and device (self-familiarize) prior to administering an injection or providing medical treatment to another person, they were allowed to do so before the simulated use portion of the session.

Test Environment	Light, furnishings and background noise streaming through a speaker simulated a typical public setting where an emergency situation may occur.
Sequence of Study	Simulated-use scenario #1 Root Cause Analysis Knowledge Assessment Tasks Root Cause Analysis Simulated-use scenario #2 (for participants who did not administer a full dose during scenario #1) Debrief and IFU Comprehension

Per the HF protocols, the age inclusion criteria for the adolescent lay caregiver user groups was 10 to 17 years of age however, the HF report states that the adolescent lay caregiver user group for the PFS study included participants 11 to 17 years of age. Per Zealand, this occurred due to difficulties recruiting participants 10 years of age for the PFS study.⁷ We find this methodology concern does not preclude our ability to review the study results.

We note that Zealand did not evaluate the tasks to roll the patient over and seek medical attention after dose administration in their HF validation studies. Zealand provided the following justification to support this methodology⁸:

...the validation study focused on whether users could safely and correctly use the product, rather than their ability to detect and correct clinical conditions... Turning the patient to their side is an additional mitigation... Seeking medical attention is an additional mitigation for the user to ensure that the patient is correctly treated.

While we disagree with Zealand regarding the purpose of their HF studies, we note similar currently marketed emergency use products with similar instructions to roll the patient over and seek medical attention. Thus, despite the lack of HF performance data for these tasks, we expect that users can comprehend that they should roll the patient over and seek medical attention after dose administration. Furthermore, our independent heuristic review of the user interface finds that the

⁷ Responses to FDA request dated 25 NOV 2020 to Human Factors Adolescent Lay Caregivers for Dasiglucagon under NDA 214231. Soborg, Denmark: Zealand Pharma A/S; 2020 DEC 02. Available from: <\\CDSesub1\evsprod\nda214231\0038\m1\us\comments-req-resp-ir-25nov2020-hf-adolescent-lay-caregivers.pdf>

⁸ Responses to FDA request dated 03 AUG 2020 to Human Factors (HF) Validation Study for Dasiglucagon under NDA 214231. Soborg, Denmark: Zealand Pharma A/S; 2020 AUG 03. Available from: <\\CDSesub1\evsprod\nda214231\0018\m1\us\comments-req-resp-ir-03aug2020-hf-validation-study.pdf>.

IFU and (b) (4) on the protective case provide clear instructions for these tasks. As such, we accept the HF validation methodology and find the residual risk acceptable for tasks related to rolling the patient over and seeking medical attention after dose administration.

We acknowledge that the HF validation study for the PFS presentation tested use related tasks (b) (4) and that subsequently, the proposed user interface was modified (b) (4). (b) (4). Based on similarity to other currently marketed products, we determined that the PFS (b) (4) does not introduce any new or unique risk in the intended user group. Thus, we determined that this modification could be implemented without additional HF testing.

3.2 RESULTS AND ANALYSES

Table 3 describes the study results, Applicant's analyses of the results, and DMEPA's analyses and recommendations.

Table 3. Identified Issues and Recommendations (Autoinjector Presentation)

	Identified Issue, Subjective Feedback, Root Cause Analysis and Mitigations	DMEPA's Analysis and Findings
1.	For the task to choose the injection site, there were 3 use errors. • One CG-AD injected into the left forearm/wrist. • One CG-AD injected into the left forearm. • One CG-AD injected into the lower back. The subjective data and Zealand's root cause analysis (RCA) indicated: • Information oversight- participants referred to the (b) (4) prior to injecting but did not note the depiction of acceptable injection sites. • Information oversight/misinterpretation- a participant misinterpreted the image of the abdomen to mean the lower back in the (b) (4) Zealand states that injection into other areas may result in intramuscular or intradermal injection, which is less ideal but acceptable routes of administration. As such, Zealand did not implement additional mitigations to further address these use errors.	Based on Zealand's use-related risk analysis (URRA), if this task is omitted or not performed correctly there is risk of minor bleeding, delayed treatment, pain, or bone strike. Thus, we are concerned with the residual risk for this error. Our review of the study results identified subjective feedback from one participant that indicated the image in the (b) (4) related to selection of the injection site may cause confusion. For example, a participant commented, "Oh that's the front! I followed the thighs and thought it was the back." Our review of the labels and labeling notes that the IFUs, (b) (4), and AI container label provides instruction to choose the injection site (i.e., "Choose site" and "Choose the injection site and remove clothing covering injection site"). We also note that the image related to the selection of the injection site task includes many elements which we find may lead to confusion when presented as proposed. For example, the image includes a human figure with clothing, arrows, and blue shaded areas. Our independent review of similar currently marketed products (e.g. Gvoke) notes that the injection sites are identified on a human figure without clothing. Based on our overall assessment, we disagree with Zealand that no additional mitigations are required to further mitigate this risk. As such, we find that the IFUs, (b) (4) and AI container label can be further improved to adequately differentiate the front from the back of the body in the image. We provide a recommendation in Table A to address this concern. We find that this change is intended to align the

		proposed labels and labeling with currently approved products. Thus, we have determined that these changes can be implemented without additional HF validation testing.
2.	For the task to expose bare skin, there were 2 use errors. - Two participants (one CG-EX and one FR) did not expose bare skin and injected through the mannequin's clothing. The subjective data and Zealand's RCA indicated: - Information oversight- both participants referred to the (b) (4) prior to injecting but did not note the highlighted exposed skin in contrast to the skin covered by clothing. Per Zealand, the clothing did not interfere with the needle in either injection (e.g., no bent or clogged needles) and the full dose was delivered. Zealand also states that the needle can penetrate most common fabrics. Thus, Zealand did not implement additional mitigations to further address these use errors.	Based on Zealand's URRR, if this task is omitted or not performed correctly there is risk of severe hypoglycemia for the patient if the needle breaks or bends or if the dose is delivered outside of the body. While we acknowledge Zealand's assertion that the needle can penetrate most clothing, we note that Zealand states, "...there is still the risk of damage to the needle and no dose administered if a user attempts to inject through clothing." Thus, we are concerned with the residual risks for this error. Our review of the subjective data identified participant feedback that indicated the lack of instruction in the (b) (4) to expose bare skin may lead users to perceive this task is a discretionary task. For example a participant stated, "It implies [in pics] with pants down, but not stated. Maybe say "must be done on bare skin" / "expose skin". Our review of the labels and labeling notes that the IFU includes the statement, (b) (4) however, the (b) (4) does not include any instruction for this task. Additionally, our independent review notes that the labeling for similar currently approved products includes instruction to expose bare skin (e.g., "choose injection site and expose skin"). Based on our overall assessment, we disagree with Zealand that no additional mitigations are required to further mitigate this risk. As such, we find that the (b) (4) and AI container label can be further improved to clarify the instructions to expose bare skin. We provide a recommendation in Table A to address this concern. We find that this change is intended to align the proposed labels and labeling with

		currently approved products. Thus, we have determined that these changes can be implemented without additional HF validation testing.
3.	For the task to remove the cap from the AI, there were 4 close calls and 1 use error. • 1 CG-AD participant failed to remove the cap before placing the needle end against the skin. • 3 CG-EX participants and 1 CG-IN participant expressed confusion about how to remove the cap, but ultimately completed the task without interference. The subjective data and Zealand’s RCA indicated: • Information oversight/labeling- participant identified the needle end but did not note the specific instruction to remove cap. • Perceptual error- a participant initially attempted to remove a cap from the white rounded end of the device but self-corrected after seeing the instruction to remove the cap. • Negative transfer- a participant relied on previous experience with an insulin pen, which does not have a cap. • Negative transfer- a participant relied on previous experience with an insulin pen that has a gray button to dispense the dose. • Mistake- a participant almost did not remove the gray cap but self-corrected.	Based on Zealand’s URRRA, if this task is omitted or not performed correctly there is risk of severe hypoglycemia for the patient. Thus, we are concerned with the residual risks for this error. We note that some of the close calls were attributed to negative transfer due to participants’ previous experience with insulin pens so we performed an independent review of the proposed device. Our assessment concluded that the AI device constituent part is similar to other currently marketed products. Thus, we agree with Zealand that no device changes are needed to address the risk that the AI may be confused with an insulin pen. However, our review of the results identified subjective feedback suggesting that the instruction to remove the AI cap on the AI container label may be overlooked. Our review of the labels and labeling notes that the AI container label includes a statement that identifies the needle end (i.e., “needle end”) and a statement that instructs users to remove the AI cap (i.e., “pull cap”). However, we find that the statement “pull cap” is less prominent than the statement “needle end”. Based on our overall assessment, we disagree with Zealand that no additional mitigations are required to further mitigate this risk. As such, we find that the AI container label can be further improved to mitigate risk of failure to remove the AI cap. We provide a recommendation in Table A to address this concern. We find that this change will not introduce any new or unique risks in the intended user group in the intended use environment. Thus, we have determined that these changes can be implemented without additional HF validation testing.

	Zealand did not implement additional mitigations to further address these use errors.	
4.	For the critical task to administer a full dose, there were 5 use errors. • 4 participants (2 CG-AD and 2 CG-IN) lifted the AI prior to administration of the full dose. • 1 CG-EX participant administered a partial dose through clothing. The subjective data and Zealand's RCA indicated: • Slip- a participant initially did not apply consistent pressure until after she felt it bounce back. The device was not lifted enough to cause the needle shield to lock back into place and the participant confirmed administration of the full dose. • Assumption- a participant assumed the injection was complete without referencing a specific indication or evidence of full dose administration. • Memory lapse- a participant intended to hold the device down for 10 seconds. • Perceptual error- a participant perceived the click and window to indicate that injection was complete. Per Zealand, in the event of removing the autoinjector from the injection site before the recommended 10 seconds, the patient will still receive a full or nearly full dose, if they ensure the window has turned red. Additionally, Zealand states	Based on the URRR, if this task is omitted or not performed correctly there is risk of severe hypoglycemia. Thus, we are concerned with the residual risks for this error. We acknowledge that Zealand did not record the specific duration of the injection time for each participant who held the AI in place for less than 10 seconds in the HF study. Our review of participant performance; specifically the use errors for this task, notes that 1 participant knew definitively that they did not administer a full dose and 4 participants were either unsure or thought the full dose was administered. We also acknowledge Zealand's assertion that patients will receive a full or nearly full dose, if the user ensures the window has turned red. However, our review of the subjective data identified participant feedback that suggests that participants misinterpreted a partially red window as full dose confirmation. Considering that 92% of the participants in the HF study indicated that they relied on the red window as an indicator for full dose delivery, we assessed the labeling to determine if any additional considerations should be taken with regard to the check window dose confirmation task. Our review of the labeling notes that the IFU, (b) (4) and container label for the AI provides 3 methods for the user to confirm full dose delivery: time (hold AI against the skin for 10 seconds), visual cue (full red window), and auditory cue (2nd click). We also note that the check window instruction is presented separately from the other dose confirmation instructions (i.e., count to 10 and 2nd click). Additionally, our independent review notes that the labeling for similar currently

	that approximately 98% of the volume of a full dose has been delivered at the second audible click. Thus, Zealand did not implement additional mitigations to further address these use errors.	approved products (e.g., Gvoke) present all dose confirmation steps as a single step. Based on our overall assessment, we disagree with Zealand that no additional mitigations are required to further mitigate this risk. As such, we find that the AI, IFU, (b) (4) and container label can be further improved to mitigate risk of failure to administer a full dose. We provide a recommendation in Table A to address this concern. We find that this change is intended to align the proposed labels and labeling with currently approved products. Thus, we have determined that these changes can be implemented without additional HF validation testing.

Table 4. Identified Issues and Recommendations (Prefilled Syringe Presentation)

	Identified Issue, Subjective Feedback, Root Cause Analysis and Mitigations	DMEPA's Analysis and Findings
1.	For the task to open the protective case, there were 3 use errors. 3 participants (CG-IN and two CG-AD) dropped the PFS while opening the protective case due to using more force than necessary and/or not holding the protective case upright. The subjective data and Zealand's RCA indicated: - Assumption- a participant expected the needle to be visible immediately upon opening the protective case as she did not consider the case as a container but possibly the device itself.	Based on the URRA, if this task is omitted or not performed correctly there is risk of severe hypoglycemia. Thus, we are concerned with the residual risks for this error. Our review of the study results notes that some participants dropped the PFS after opening the protective case due to incorrect orientation of the protective case while performing this task. Additionally, we identified participant performance that suggests the instructions related to opening the protective case in the (b) (4) can be improved to present this information with other important administration/use instructions. Our review of the labels and labeling notes that the location and horizontal orientation of the instruction related to this task differs from the (b) (4) for the AI presentation. We are concerned that as proposed,

	• Information Oversight- a participant did not note or understand that the PFS was contained within the protective case. • Information Oversight- a participant did not note the instruction about how to hold the protective case when opening as she was more concerned about simply getting it open to administer the medication. Per Zealand, based on participant feedback from the formative study, the protective case was redesigned to be opened more easily and the (b) (4) was revised to show users how to open the protective case with written instructions and a visual aid. Additionally, the protective case cap is colored gray to cue users how to open the protective case and the (b) (4) and IFU include instructions and a visual aid for how to open the protective case. Thus, Zealand determined that these use errors are not amenable to additional device or labeling mitigations.	the presentation of this instruction on the (b) (4) for the PFS may lead to confusion because the protective case should be held vertically while performing this task to minimize damage to the PFS. Based on our overall assessment, we disagree with Zealand that no additional mitigations are required to further mitigate this risk. As such, we find that the (b) (4) for the PFS can be further improved to mitigate risk of damage to the PFS while opening the protective case. We provide a recommendation in Table A to address this concern. We find that this change will not introduce any new or unique risk in the intended user group in the intended use environment. Thus, we have determined that these changes can be implemented without additional HF validation testing.
2.	For the task of exposing bare skin, there was 1 use error and 2 instances of study artifact. • 1 CG-AD participant administered the injection through clothing. • 2 participants (1 CG-EX and 1 CG-IN) stated that they would have exposed bare skin if they were injecting into a real person and not a mannequin. The subjective data and Zealand’s RCA indicated:	Based on the URRRA, if this task is omitted or not performed correctly there is risk of severe hypoglycemia. While we acknowledge Zealand’s assertion that the needle can penetrate most clothing, we note that Zealand states, “...there is still the risk of damage to the needle and no dose administered if a user attempts to inject through clothing.” Thus, we are concerned with the residual risks for this error. Our review of the subjective data identified participant feedback that indicated the lack of instruction in the (b) (4) to expose bare skin may lead users to perceive this as a discretionary task. For example a participant stated, “It’s pictured but it’s not specific. It doesn’t

• Lapse- participant understood the need to move clothing away from the injection site to allow needle insertion directly into the skin but did not do so due to memory failure. • Study artifact- participants stated that they would have exposed bare skin if they were injecting into a real person and not a mannequin. Per Zealand, the needle gauge allows for injection through most clothing. Zealand also states that after the formative study, additional labeling was added to the (b) (4) to include an image of the injection sites on bare skin. The images depicting the injection into skin were also increased in size to enhance readability. Thus, Zealand determined that these use errors are not amenable to additional device or labeling mitigations.	specifically say it.” Our review of the labels and labeling notes that the IFU includes the statement, (b) (4) however, the (b) (4) does not include any instruction for this task. Additionally, our independent review notes that the labeling for similar currently approved products includes instruction to expose bare skin (e.g., “choose injection site and expose skin”). See #2 in Table 3 above for our overall assessment and findings.

3.3 ANALYSIS OF OTHER TASK ERRORS

The HF validation studies showed use errors, (e.g. failures, difficulties, and close calls) with the following six critical tasks and three non-critical tasks, however our assessment of these user errors finds the residual risk is acceptable and thus are not the focus of this review. We reviewed the available participants' subjective feedback, the Applicant's root cause analysis and Applicant's proposed risk mitigation strategy to determine acceptability. In addition, we considered the use tasks of the proposed product with the use tasks in similar marketed products with the same user groups to determine if there are any current concerns of vulnerability to use error. Subsequently, our assessment of the aforementioned considerations in totality finds the residual risk is acceptable for the use tasks below; thus, we find no recommendations to further address the use errors or mitigations are necessary at this time to address the use errors related to the following use tasks:

- Identify the correct product (AI, PFS)
- Open the protective case (AI)
- Place the needle end (needle guard) towards injection site (AI)
- Administer the full dose (PFS)
- Pinch the skin (PFS)
- Insert the needle at a 45 degree angle (PFS)
- Remove the needle at the insertion angle (PFS)
- Keep out of reach of children (AI, PFS)
- Dispose in a sharps container (AI, PFS)
- Discard the AI cap (AI)
- Dispose of the expired product after the expiration date (AI, PFS)
- Do not administer if damaged (AI, PFS)
- Difference between both expiration dates (AI, PFS)
- Remove the needle cover (PFS)
- Proper storage conditions (AI, PFS)
- Disposal of the rigid needle shield (PFS)

3.4 LABELS AND LABELING

Tables 5 and A below include the identified medication error issues with the submitted labels and labeling, our rationale for concern, and the proposed recommendation to minimize the risk for medication error.

Table 5: Identified Issues and Recommendations for Division of Diabetes, Lipid Disorders, and Obesity			
	Identified Issue	Rationale for Concern	Recommendation
General Issues			
1.	The placeholder “Proprietary Name”, is used throughout the PI.	The proposed proprietary name, Zegalogue, was found conditionally acceptable on May 6, 2020.	Replace “Proprietary Name” with the conditionally approved proprietary name, “Zegalogue” throughout the PI.
Full Prescribing Information: Section 2 Dosage and Administration			
2.	Subsections 2.1 and 2.2 state that the (b) (4) is an appropriate injection site; however, the IFU states “lower abdomen”.	We are concerned that inconsistent information regarding the appropriate injection site may lead to wrong technique medication error.	Revise the term (b) (4) to “lower abdomen” in subsections 2.1 and 2.2.
Full Prescribing Information: Section 16 How Supplied/ Storage and Handling			
3.	The presence of the warning statement, (b) (4)	Post-marketing reports that negative statements (e.g. do not) may have the opposite of the intended meaning because the word (b) (4) can be overlooked and the warning may be misinterpreted as an affirmative action. ⁹	Revise the statement, (b) (4) to “Discard after...whichever occurs first.”

⁹ Institute for Safe Medication Practices. Affirmative warnings (do this) may be better understood than negative warnings (do not do that). ISMP Med Saf Alert Acute Care. 2010;15(16):1-3.

4.	The NDC numbers are not included in Section 16.1.	We are unable to evaluate the NDC numbers from a medication error perspective.	Add the intended NDC numbers and submit for Agency review.
Full Prescribing Information: Section 3 Dosage Forms and Strengths, Section 16 How Supplied/ Storage and Handling			
5.	The description of your device constituent parts can be improved. The term (b) (4) lacks clarity. Additionally, the prefilled syringe presentation is described as a (b) (4); however, it does not include a (b) (4) device.	We are concerned that the terms (b) (4) may not facilitate identification of your proposed product which may lead to confusion.	1. Revise (b) (4) to (b) (4) autoinjector". 2. Revise (b) (4) to "syringe".
Full Prescribing Information: Section 17 Patient Counseling Information			
6.	Advising patients, family members, or caregivers of the symptoms of (b) (4) hypoglycemia is inconsistent with the proposed indication (i.e., "for the treatment of severe hypoglycemia).	We are concerned that lack of clarity regarding the intended use of your product may cause confusion which may lead to patient harm.	Remove (b) (4)

Table A: Identified Issues and Recommendations for Zealand Pharma A/S (entire table to be conveyed to Applicant)			
	Identified Issue	Rationale for Concern	Recommendation
General (All Labels and Labeling)			
1.	The labels and labeling present (b) (4) as the proprietary name.	We reference our May 6, 2020 letter informing you that the proprietary name, Zegalogue, was found conditionally acceptable.	Remove all references to "(b) (4)" and replace with the conditionally acceptable proprietary name, "Zegalogue."
2.	The strength statement is presented as "0.6mg/0.6mL."	Including a space between the numeral and unit of measurement improves the readability of the product's strength.	To improve readability, place adequate space between the numerical dose and unit of measure (e.g. 0.6 mg/0.6 mL).
3.	As presented, the dosage form is not located immediately after the established name.	The dosage form, injection, should be included either on the same line as the established name or directly below the established name.	Revise the established name and dosage form to read as follows: "Zegalogue (dasiglucagon) injection."
Instructions for Use (IFU) for the Autoinjector (AI) and Prefilled Syringe (PFS)			
4.	As presented, the storage statement lacks prominence.	We are concerned that the lack of prominence may lead to deteriorated drug error.	Revise and bold the statement to "Store Zegalogue in the refrigerator between 2°C to 8°C ((b) (4) F to 46°F)". We recommend this to increase the prominence of this important information and minimize the risk of the storage information being overlooked.
IFU and (b) (4) Protective Case Label for the AI and PFS and Container Label for the AI			

5.	Your IFUs, (b) (4), and AI container label can be improved to better identify the appropriate injection sites. In your HF study, a participant had difficulty interpreting the image used to depict the injection sites and injected into the lower back.	We are concerned that as presented, the images related to the selection of the injection site task may cause misinterpretation, which may lead to selection of the wrong injection site.	Revise the image related to the selection of the injection site to: 1. Remove the clothing from the human in the image (i.e., highlight the injection sites on an unclothed body). 2. Include labels to further differentiate the front from the back of the human in the image (e.g., include words "front" and "back").
IFU, (b) (4) and Container Label for the AI			
6.	Your IFU, (b) (4) and container label for the AI can be improved to better present all steps related to dose confirmation. We note that you state in the event of removing the autoinjector from the injection site before the recommended 10 seconds, the patient will still receive a full or nearly full dose, if they ensure the window has turned red. However, in your HF study, some participants removed the AI from the skin before the dose confirmation window was entirely red. Also, we note that your IFU, (b) (4) and AI container label present the "check window" instruction as a step after the other dose	We are concerned that the lack of clarity in the instruction for the dose confirmation task may lead to underdose error. We acknowledge that your root cause analysis attributed slip, assumption, and memory lapse for the use issues observed for the dose confirmation task in your HF study for the AI. However, our review identified participant feedback that suggests misinterpretation of a partially red window as full dose confirmation. For example, "I didn't hold for the full 10 seconds but I held until [window] turned red." Additionally, based on the currently presented steps, we are concerned that users may remove the	Revise your IFU, (b) (4), and container label for the AI to present all steps related to dose confirmation (i.e., count to 10, 2 nd click, full red window) as a single task to convey that the AI should not be removed from the skin until after the window is entirely red. For example, consider combining the "Push...10 seconds" and "Check window...remove" step into a single step.

	confirmation steps (i.e., count to 10 and 2nd click).	AI from the skin after 10 seconds and after the 2nd click but before the window is entirely red which may lead to underdose error.	
(b) (4)			
Container Labels and Carton Labeling for the AI and PFS			
5.	The format of the human-readable portion of the product identifier is not identified. Additionally, the	The Drug Supply Chain Security Act (DSCSA) requires, for certain prescription products, that the	The DSCSA guidance on product identifiers recommends a machine-readable (2D data

	carton labeling for the 2 pack presentations for the AI and PFS do not include a machine-readable (2D data matrix barcode) product identifier.	smallest saleable unit display a human-readable and machine-readable (2D data matrix barcode) product identifier.	matrix barcode) product identifier and a human-readable product identifier. Include the machine-readable data matrix barcode to the carton labeling. The guidance also recommends the format of the human-readable portion be located near the 2D data matrix barcode as the following: NDC: [insert NDC] SERIAL: [insert serial number] LOT: [insert lot number] EXP: [insert expiration date] We recommend that you review the draft guidance to determine if the product identifier requirements apply to your product's labeling. The draft guidance is available from: https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm621044.pdf .
Container Label for the PFS			
6.	The linear barcode has been omitted.	The drug barcode is often used as an additional verification before drug administration in the hospital setting;	We request you add the product's linear barcode to each individual prefilled syringe as required per 21CFR

		therefore, it is an important safety feature that should be part of the label whenever possible.	201.25(c)(2). We recommend orienting the linear barcode to a vertical position to improve the scannability of the barcode. Barcodes placed in a horizontal position may not scan due to curvature of the PFS. ¹⁰
Container Label for the AI			
7.	The AI container label can be further improved to mitigate risk of failure to remove the AI cap prior to administration. The subjective data and your root cause analysis indicated that the instruction to remove the cap lacks prominence.	We are concerned that this important instruction may be overlooked and result in dose omission or a delay in therapy.	Revise the AI container label to increase the font size of the statement, "Pull Cap" so that it appears equally prominent as the statement, "Needle End".
Carton Labeling for the AI and PFS			
8.	Your carton labeling contains redundant information which may contribute to clutter. Specifically, the statement, (b) (4) appears on the principal display panel (PDP) and on a lower panel.	We are concerned that this redundant statement may compete with critical drug information on the PDP such as the proprietary and established names, and strength.	Remove the statement, (b) (4) from the PDP.
9.	The carton labeling for the 2 pack presentations for the AI and PFS include an overly prominent net quantity statement (i.e., "2 pack").	The prominence of the net quantity statement draws attention away from important product identifying information such as the proprietary	Decrease the prominence of the net quantity statement, "2 pack", on the carton labeling for the 2 pack presentations for the AI and PFS.

¹⁰ Neuenschwander M. et al. Practical guide to bar coding for patient medication safety. Am J Health Syst Pharm. 2003 Apr 15;60(8):768-79.

		name, established name, and strength.	
Container Labels and Carton Labeling for the AI and PFS			
10.	As currently presented, the National Drug Code (NDC) number is not included on carton labels and container labeling.	Per 21 CFR 207.33, drug products subject to listing with the FDA must have a unique NDC to identify its labeler, product, and package size and type.	Add the intended NDC numbers on the container labels and carton labeling and submit for our review.
11.	The format for the expiration date is presented inconsistently throughout labels and labeling.	Lack of clarity regarding the expiration date might contribute to confusion and deteriorated drug medication errors.	Confirm whether the expiration date will be consistently expressed as "MMYYYY" throughout labels and labeling and if alphabetical letters will be used to represent the month.

4. CONCLUSION AND RECOMMENDATIONS

Our review of the results of the human factors (HF) validation study results identified use errors with critical tasks. However, taking into consideration the review of the subjective feedback, root cause analysis, and our independent review of similar currently approved products, we find residual risks associated with these use errors acceptable. Thus, in this specific instance, we accept the simulated HF validation study results.

Our evaluation of the proposed packaging, label and labeling identified areas of vulnerability that may lead to medication errors. Above, we have provided recommendations in Table 5 for the Division and Table A for the Applicant. We ask that the Division convey Table A in its entirety to the Applicant so that recommendations are implemented prior to approval of this NDA.

4.1 RECOMMENDATIONS FOR THE ZEALAND

We find the results of your human factors (HF) validation studies acceptable. However, our evaluation of the results of your human factors (HF) validation study, including participant subjective feedback and root cause analyses, indicates that there are additional mitigations that can be implemented to address some of the use errors that occurred with critical tasks. Additionally, our review of the labels and labeling identified areas of vulnerability that may lead to medication errors. We provide these recommendations in Table A and we recommend that you implement these recommendations prior to approval of this NDA.

APPENDICES: METHODS & RESULTS FOR EACH MATERIALS REVIEWED

APPENDIX A. DRUG PRODUCT INFORMATION/PRESCRIBING INFORMATION

Table 5 presents relevant product information for Zegalogue that Zealand submitted on March 27, 2020 and September 30, 2020.

Table 5. Relevant Product Information	
Initial Approval Date	N/A
Therapeutic Drug Class or New Drug Class	antihypoglycemic agent
Active Ingredient (Drug or Biologic)	dasiglucagon
Indication	for treatment of severe hypoglycemia in patients with diabetes aged 6 years and above
Route of Administration	subcutaneous
Dosage Form	injection
Strength	0.6 mg/0.6 mL
Dose and Frequency	0.6 mg administered by subcutaneous injection into the abdomen, buttocks, thigh, or upper arm.
How Supplied	supplied as a ready-to-use, preservative free, aqueous, clear, colorless solution for subcutaneous injection in the following single-use presentations: • 1 HypoPal Autoinjector • 2 HypoPal Autoinjectors • 1 Prefilled syringe • 2 Prefilled syringes
Storage	Store in a refrigerator, 2°C to 8°C (36°F to 46°F). Keep away from the cooling element. During the shelf-life, Zegalogue can be kept at room temperature up to 25°C [77°F]) for a single period of up to 12 months. Do not return the product to the refrigerator after storing at room temperature. Do not freeze. Protect from light. Store in the provided protective case.
Container Closure/Device Constituent	• Single-dose prefilled HypoPal autoinjector • Single-dose prefilled safety syringe
Intended Users	first responders (emergency medical services (EMS), including paramedics), adult lay caregivers of patients with diabetes, adults and adolescents with close or familial ties to patients with diabetes, or adolescent lay users with close or familial ties to someone with diabetes

Intended Use Environment	any public or private, indoor or outdoor setting in which a medical emergency may occur

APPENDIX B. BACKGROUND INFORMATION

B.1 PREVIOUS HF REVIEWS

B.1.1 Methods

On April 15, 2020, we searched the L:drive and AIMS using the terms, IND 127866 and NDA 214231 to identify reviews previously performed by DMEPA or CDRH.

B.1.2 Results

Our search identified 2 previous reviews^{3,11}, and we considered our previous recommendations to see if they are applicable for this current review.

APPENDIX C. BACKGROUND INFORMATION ON HUMAN FACTORS ENGINEERING PROCESS

The background information can be accessible in the HF results report. See Appendix D.

APPENDIX D. HUMAN FACTORS VALIDATION STUDY RESULTS REPORT

The HF study results report for the autoinjector presentation can be accessible in EDR via: <\\cdsesub1\evsprod\nda214231\0001\m5\53-clin-stud-rep\535-rep-effic-safety-stud\hypoglycemia\5354-other-stud-rep\rep-5344\rep-5344-hfe-ai.pdf>

The HF study results report for the prefilled syringe presentation can be accessible in EDR via: <\\cdsesub1\evsprod\nda214231\0001\m5\53-clin-stud-rep\535-rep-effic-safety-stud\hypoglycemia\5354-other-stud-rep\rep-5343\rep-5343-hfe-pfss.pdf>

APPENDIX E. INFORMATION REQUESTS ISSUED DURING THE REVIEW

On May 6, 2020, we issued an Information Request (IR) to request a list of currently marketed products that use the same rigid needle shield and (b) (4) as the proposed product.

Our IR and the Applicant's response can be accessible in EDR via:

<\\cdsesub1\evsprod\nda214231\0005\m1\us\comments-req-response-syringe-components-06may2020.pdf>

On June 5, 2020, we issued an IR to request the missing elements listed below for each use error for the knowledge tasks observed in the HF.

- description of each knowledge task use error (i.e., answer provided by each participant)

¹¹ Little. C. Human Factors Validation Study Protocol Review for dasiglucagon IND 127866. Silver Spring (MD): FDA, CDER, OSE, DMEPA (US); 2019 JUN 13. RCM No.: 2019-861.

- subjective feedback
- root cause analysis
- discussion of mitigation strategies

The Applicant's response can be accessible in EDR via:

Autoinjector HF study report addendum: <\\cdsesub1\evsprod\nda214231\0011\m5\53-clin-stud-rep\535-rep-effic-safety-stud\hypoglycemia\5354-other-stud-rep\rep-5344\rep-5344-hfe-ai-addendum.pdf>

Prefilled syringe HF study report addendum: <\\cdsesub1\evsprod\nda214231\0011\m5\53-clin-stud-rep\535-rep-effic-safety-stud\hypoglycemia\5354-other-stud-rep\rep-5343\rep-5343-hfe-pfss-addendum.pdf>

On June 9, 2020, we issued an IR to request:

- justification for Zealand's determination that additional mitigation strategies are not needed to address the residual risk to intended users (and subsequently patients) who do not safely and effectively perform the task of securing the needle (b) (4) to minimize the risk of a needle stick injury.
- discussion on whether use errors and close calls related to the removal of the RNS task (or any other critical task failures) were attributed to the (b) (4) (b) (4) proposed user interface.

Our IR and the Applicant's response can be accessible in EDR via:

<\\cdsesub1\evsprod\nda214231\0010\m1\us\comments-req-resp-ir-09jun2020-pfss-rigid-shield.pdf> (b) (4)

On June 12, 2020, we issued an IR to request:

- The specific duration of time for each participant who held the autoinjector in place for less than 10 seconds.
- The volume of medication delivered at each visual and audible cue.
- The actual time required to hold the device in place after the first click to ensure a complete dose is delivered.

Our IR and the Applicant's response can be accessible in EDR via:

<\\cdsesub1\evsprod\nda214231\0012\m1\us\comments-req-resp-ir-12jun2020-hf-ai-injection-time.pdf>

On August 3, 2020, we issued an IR to request:

- Zealand's justification for not evaluating these critical tasks in your HF validation studies.

- Discussion of whether additional risk mitigation measures are necessary to address observed use errors for the choose the injection site task
- Additional discussion regarding observations 004-026 CG IN, 002-008 CG IN, and 004-016 CG EX in the HF validation study for the auto-injector presentation.

Our IR and the Applicant's response can be accessible in EDR via:

<\\CDSESUB1\evsprod\nda214231\0018\m1\us\comments-req-resp-ir-03aug2020-hf-validation-study.pdf>

Autoinjector HF study report Addendum: <\\CDSESUB1\evsprod\nda214231\0018\m5\53-clin-stud-rep\535-rep-effic-safety-stud\hypoglycemia\5354-other-stud-rep\rep-5344\rep-5344-hfe-ai-addendum-2.pdf>

Prefilled syringe HF study report Addendum: <\\CDSESUB1\evsprod\nda214231\0018\m5\53-clin-stud-rep\535-rep-effic-safety-stud\hypoglycemia\5354-other-stud-rep\rep-5343\rep-5343-hfe-pfss-addendum-2.pdf>

On October 8, 2020, we issued an IR to request the proposed intend-to-market protective case label for the prefilled syringe presentation.

Our IR and the Applicant's response can be accessible in DARRTS via:

https://darrts.fda.gov//darrts/faces/ViewDocument?documentId=090140af8059f3c3&_afRedirect=1163461702473755

On November 25, 2020, we issued an IR to request Zealand's justification for not including 10 year old adolescent participants in the HF validation study for the PFS.

Our IR and the Applicant's response can be accessible in EDR via:

<\\CDSESUB1\evsprod\nda214231\0038\m1\us\comments-req-resp-ir-25nov2020-hf-adolescent-lay-caregivers.pdf>

APPENDIX F. LABELS AND LABELING

E.1 List of Labels and Labeling Reviewed

Using the principles of human factors and Failure Mode and Effects Analysis,¹² along with postmarket medication error data, we reviewed the following Zegalogue labels and labeling submitted by Zealand.

- Autoinjector Container Labels received on March 27, 2020

¹² Institute for Healthcare Improvement (IHI). Failure Modes and Effects Analysis. Boston. IHI:2004.

- Autoinjector Protective Case Label received on March 27, 2020
- Autoinjector Carton labeling received on March 27, 2020
- Prefilled syringe Container Labels received on September 30, 2020
- Prefilled syringe Carton labeling (1 pack) received on March 27, 2020
- Prefilled syringe Carton labeling (2 pack) received on September 30, 2020
- Prefilled syringe Protective Case Label (front) received on September 30, 2020
- Prefilled syringe Protective Case Label (back) received on March 27, 2020
- Autoinjector Instructions for Use received on March 27, 2020. Available from <\\cdsesub1\evsprod\nda214231\0001\m1\us\instructions-for-use-ai.pdf>
- Prefilled syringe Instructions for Use received on September 30, 2020. Available from <\\CDSESUB1\evsprod\nda214231\0027\m1\us\instructions-for-use-pfss.pdf>
- Prescribing Information (Image not shown) received on March 27, 2020. Available from <\\cdsesub1\evsprod\nda214231\0001\m1\us\draft-annot-labeling-text-prescribing-information.docx>

E.2 Label and Labeling Images

Autoinjector Container Labels



This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

COLLEEN L LITTLE
12/10/2020 06:31:11 PM

LOLITA G WHITE
12/11/2020 12:44:44 PM

JASON A FLINT
12/11/2020 12:50:37 PM

MISHALE P MISTRY
12/11/2020 12:53:18 PM

Center for Drug Evaluation and Research
Office of Biotechnology Products
Division of Biotechnology Research and Review II



Proposed regulatory pathway: NDA 505(b)(1)

NDA: NDA 214231, SDN 0001, dated March 27, 2020
and SDN 0016, dated June 24, 2020 safety update.

Drug Name: Dasiglucagon, formerly ZP4207

Proposed proprietary name: Zegalogue®

Primary Reviewer: Faruk Sheikh, Ph.D., Chemist, OBP, DBRR-II, CDER

Secondary Reviewer: Harold Dickensheets, Ph.D., Chemist, OBP, DBRR-II, CDER

Tertiary Reviewer: Patrick Lynch, Ph.D., Lead Biologist, OBP, DBRR-II, CDER

Therapeutic dose: 0.6 mg/0.6 mL (1mg/mL strength)

Presentation: Ready-to-use aqueous formulation in a pre-filled syringe and an autoinjector

Proposed Indication: Severe hypoglycemia in diabetic patients aged 6 years and above

Sponsor: Zealand Pharma, Copenhagen, Denmark

Project Manager: Liz Godwin

Internal mid-cycle meeting: August 11, 2020

Primary review due: November 27, 2020

Secondary review due: December 4, 2020

PDUFA Goal Date: Saturday, March 27, 2021

1. Recommendation:

The sponsor submitted immunogenicity assessment data from eleven clinical studies including one pivotal, multi-dose phase 3 immunogenicity study (#ZP4207-16136) in support of the immunogenicity of dasiglucagon drug product to be used in rescue treatment of diabetic patients with severe hypoglycemia. The immunogenicity assays used to evaluate the development of antibodies to dasiglucagon under this NDA are appropriately validated. No safety concern could be linked to the low-incidence of ADA development from dasiglucagon trials under this NDA. I recommend approval of this product from immunogenicity perspective.

2. Executive summary:

Dasiglucagon or Zegalogue (sponsor-proposed) is developed as rescue treatment option for severe hypoglycemia in adults and pediatric (age 6 or older) patients with diabetes mellitus and supplied as ready-to-use aqueous formulation at 1 mg/mL in a pre-filled syringe and an autoinjector.

In support of the immunogenicity of dasiglucagon under this NDA 213241 application, the sponsor submitted immunogenicity assessment data generated from 11 clinical trials by using validated immunogenicity assays, where 498 subjects with type 1 diabetes mellitus (T1DM) were exposed to single or multiple doses of dasiglucagon and 212 subjects were exposed to glucagon. Four of 498 patients (0.8%) who received dasiglucagon during the clinical trial developed anti-drug antibodies, while one of 212 patients (0.5%) receiving glucagon (GlucaGen) developed antibodies to glucagon. The four anti-dasiglucagon antibody (ADA)-positive patients included two subjects from single-dose trials, one from a dose-finding cross-over trials of single dose of dasiglucagon and glucagon and one from a supportive multi-dose cross-over trial of dasiglucagon and glucagon (b) (4).

The ADA-positive (ADA+) subject from multi-dose cross-over study received 8 doses of dasiglucagon and 3 doses of glucagon over a 21-day treatment period via a pump device. This subject tested ADA+ approximately 6 weeks after first dose, showed cross-reactivity with glucagon and also tested positive for dasiglucagon and glucagon neutralizing antibody (NAb) activity using *in vitro* assays. A subsequent sample at 3.5 months was ADA+ but NAb negative. The impact of the neutralizing activity of the antibodies in this sample on safety and efficacy is unclear at this time. There is no indication that the NAb had an effect on PK or PD. Development of high ADA titers was not observed. No other ADA+ subjects developed NAb responses during the treatment and follow-up periods, suggesting that either patient-specific factors, multiple doses and/or the drug switching protocol may have been responsible for the observed NAb development.

In the phase 3 pivotal immunogenicity trial (Study ZP4207-16136), none of the patients receiving weekly subcutaneous doses of dasiglucagon (n=57), glucagon (n=54) or placebo for three weeks developed antibodies to dasiglucagon or glucagon through 15 weeks of first dose.

The immunogenicity data from 11 clinical studies suggest that the overall incidence of immunogenicity of dasiglucagon is low (<1%; 4 of 498 subjects). Due to the small number of ADA+ patients observed, statistical analysis for the impact of ADA formation on PK, PD, efficacy and/or safety was not performed. Instead, the sponsor provided individual safety or efficacy data for these four ADA+ patients, and no negative impact could be correlated with ADA development in these ADA+ patients, including the single NAb+ subject. In my opinion there may be insufficient data available to fully evaluate the impact of ADA on safety or performance of the drug. Nevertheless, while the immunogenicity risk for the proposed single-dose dasiglucagon rescue treatment indication in diabetic patients with severe hypoglycemia may be expected to be low, the available data may not be fully predictive for multiple-dose, long-term therapy indications.

Reviewer's note on the validation of immunogenicity assays: The sponsor submitted dasiglucagon and glucagon screening and confirmatory assay validation reports as well as the

NAb assays (b) (6) on May 27, 2016. During the course of the development of dasiglucagon drug (b) (6) the previous OBP immunogenicity assessor reviewed the validation of immunogenicity assays and requested additional information needed to complete the review (DARRTS entry on Oct 10, 2018). The response to that request was not found while evaluating the status of the immunogenicity assay validations during pre-NDA review. Upon contacting Zealand Pharma, they acknowledged, in an email correspondence in October 2019 to the RPM Elizabeth Godwin, that the response to that letter was not formally submitted. We recommended the sponsor to resubmit the complete validation packages for all immunogenicity assays including the response to missing 'information request' letter, during the submission of the new NDA application (DARRTS Reference ID: 4535643, 12/19/2019). The sponsor submitted complete reports related to immunogenicity assays to the new NDA 214231.

On June 19, 2020, after initial review of the immunogenicity assay validation reports, OBP requested additional information. On June 26, 2020, the sponsor provided responses to our comments (SDN 0013), which were reviewed. It was subsequently determined that the assay validations are complete and the validated assays are suitable for their intended purpose and may be used in clinical sample analysis ([Appendix 1](#) and [Appendix 2](#)). These assays include the anti-dasiglucagon antibody binding assay, anti-glucagon antibody binding assay, cross-reactivity and titering assay for analyzing antibodies to dasiglucagon and neutralizing antibody assays for antibodies to dasiglucagon and glucagon.

Review memorandum

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1. Introduction:

Zealand Pharma submitted this New Drug Application (NDA) for dasiglucagon injection on March 27, 2020 seeking marketing approval (proposed proprietary name Zegalogue[®]) under 505(b)(1) of the Federal Food Drug and Cosmetic Act. This ready-to-use aqueous formulation of dasiglucagon is intended to use for the treatment of severe hypoglycemia to be administered as a single-dose subcutaneous (SC) injection in patients with diabetes ages 6 years and above. The drug is developed in two presentations for commercialization: 0.6 mg/mL in a pre-filled syringe and 0.6 mg/mL in an auto-injector (HypoPal[®]).

Dasiglucagon is a glucagon receptor (GR) agonist, which binds to GR and induces liver glycogen breakdown process, releasing glucose from the liver, resulting to an increase in blood glucose levels in severe hypoglycemic patients. Dasiglucagon is synthesized based on 29 amino acids of human native glucagon sequence (b) (4)

(b) (4) The amino acid modifications are shown in black fonts in Fig1, below.

The modifications in dasiglucagon are expected to (b) (4)

Fig 1: Dasiglucagon and human glucagon amino acid sequence comparison: (b) (4)

2. Immunogenicity risk analysis of dasiglucagon administration:

The *in-silico* analysis for immunogenicity potential of dasiglucagon performed by the sponsor indicated that the predicted risk was low. However, the *in-silico* algorithm was not able to include the (b) (4) amino acid of dasiglucagon, hence, the immunogenicity potential of dasiglucagon cannot be explained entirely by *in-silico* data analysis.

In general, modifications in peptide sequence may increase the risk of generating a new immune response in comparison to native versions. The sponsor's (b) (4) may potentially increase immunogenic risk since (b) (4) is known to be stereochemically constrained and may cause local helical conformations leading the dasiglucagon peptide to adopt a different secondary structure than non-substituted endogenous versions. However, the use of (b) (4) in

¹ Tsuchiya K and Numata K (2017): Chemoenzymatic synthesis of polypeptides containing the unnatural amino acid 2-aminoisobutyric acid. *Chemical Communications*, 53, 7318--7321.

semaglutide-O (oral tablet), a GLP-1 analog approved by FDA in September 2019 demonstrated to be mildly immunogenic (1.4% subjects developed ADAs, Ref: drug label).

Immunogenicity is known to be influenced by fibrils and aggregate formation (such as subvisible particulates, multimers) in other glucagon drugs, and may potentially accumulate under the in-use conditions for dasiglucagon injection. To prevent such formation, the sponsor designed dasiglucagon

(b) (4)

(b) (4)

The *potential* that dasiglucagon may elicit the development of ADAs if the drug is required to be used more than once to counter hypoglycemia in patients cannot be fully excluded by the currently available data. If ADA is observed in such situation, these ADA may potentially neutralize the drug or with wider implications cross-react with and neutralize endogenous glucagon. Therefore, anti-dasiglucagon ADA may potentially affect not only safety and efficacy of the product, but potentially affect the subject's glucose metabolism. If formation of high-titer antibodies is observed in the future (post-approval), this could also theoretically influence the efficacy of dasiglucagon and glucagon either indirectly by affecting the PK or directly by neutralizing the intended response to dasiglucagon.

It should be noted that the potential effects noted above were not demonstrated in the limited number of ADA+ subjects found in the sponsor's studies. Further, the average half-life of dasiglucagon is 28 minutes and it is cleared from the circulation via endogenous proteolytic pathways. Therefore, the possibility of an effective immune response is expected to be low from a proposed single dose application of dasiglucagon.

3. Immunogenicity experience from glucagon administration:

Several Glucagon products are currently approved to use as rescue medication for injection (GlucaGen[®] by Novo Nordisk; Glucagon[®] by Eli Lilly and Gvoke by Xeris) and also for nasal administration (Baqsimi[®] by Eli Lilly).

No glucagon-specific antibodies are known to develop from subcutaneous injection of currently approved glucagon (Eli Lilly, NDA 20-928; Novo Nordisk, NDA 20-918). However, according to the Baqsimi label, 2% (3/124) of treated patients administered through the nasal inhalation route developed treatment-emergent anti-drug antibodies to glucagon. Since no prior clinical experience is available with dasiglucagon, risk of ADA development to dasiglucagon was evaluated to inform the potential risk of ADA to dasiglucagon. It is generally thought that a

² Pedersen JS, Dikov D, Otzen DE (2006) N- and C-terminal hydrophobic patches are involved in fibrillation of glucagon. *Biochemistry*;45:14503-12.

lower incidence of ADA development is known in patients administered with drugs which have shown high degree of amino acid sequence homology to an in vivo counterpart, most likely due to immunologic tolerance. Dasiglucagon shows about 75% amino acid sequence homology with native human glucagon, therefore, the ADA development potential of dasiglucagon may be expected to be low.

4. Immunogenicity assays:

Zealand developed and validated two antibody binding and confirmatory assays; one for the detection of anti-dasiglucagon ADA and another for detection of anti-glucagon ADA, in dasiglucagon- and glucagon-treated subjects, respectively. In addition to antibody binding assays, Zealand also plans to use the anti-glucagon ADA assay to determine if anti-dasiglucagon antibody positive samples are cross-reactive to endogenous glucagon. The cross-reactive ADAs will be further characterized for their ability to neutralize glucagon activity by an anti-glucagon neutralizing antibody (NAb) assay. Zealand also validated an anti-dasiglucagon NAb assay to determine if the anti-dasiglucagon ADAs can inhibit the function of dasiglucagon.

The binding and NAb assays were reviewed earlier (DARRTS Ref: 4332386, dated 10/10/2018). Zealand resubmitted complete assay validation reports for anti-ZP4207 and anti-glucagon antibody binding assay, anti-ZP4207 NAb and anti-glucagon NAb assays in this NDA, as requested (DARRTS Reference ID: 4533848, 12/13/2019). The assay validation reports are determined to be acceptable and are briefly summarized below.

4.1. Anti-drug antibody screening and confirmatory assays:

The anti-dasiglucagon antibody binding assay (Study number: 14-173) and anti-glucagon antibody binding assay (Study number: 14-074) submitted to IND (b) (4) were reviewed by OBP immunogenicity reviewer, Mohan Manangeeswaran, Division III (DARRTS Ref: 4332386, dated 10/10/2018). The validation review was revisited and completed in [Appendix 1](#) of this memorandum.

The assay cut-points were validated using serum samples from healthy individuals and the validated cut-points (CP) were later verified using pre-dose serum samples from T1DM trial subjects to determine the need for use of an in-study CP for each trial separately (DARRTS Ref: 4366058, 12/19/2018 submitted to IND (b) (4) SDN 014).

4.1.1 Anti-ZP4207 antibody binding (BAb) assay:

- For anti-ZP4207 ADA screening assay, the plate wells (96-well) were coated with ZP4207.
 - Positive control antibody is a mouse monoclonal antibody (Mab3118-M-F1-4, Lot No. MAb3118-M-F1-4-t3), further details about the antibody is provided in [Table 1](#).
 - Assay method= ELISA
 - Secondary detection reagent is anti-mouse IgG HRP conjugate.

- Screening Cut-Point (SCP): Serum samples from 40 healthy subjects were used to determine a normalization factor (NF) = 0.1635 (validation study 14-173).

[Reviewer Note: Zealand used pre-dose serum samples from thirty (30) T1DM subjects from pivotal immunogenicity trial ZP4207-16136³, and determined a normalization factor (NF) of 0.10035 to use in the calculation of the plate specific cut-point (PSCP) for use in subsequent clinical batches (Study number: 18-077; (b)(4)/115, partial validation report). Additional human IgG and IgM controls were used in this assay as suggested by the FDA to demonstrate the suitability of the assay secondary detection reagent (anti-mouse IgG conjugate) to detect human Ig isotypes, and to ensure that assay is capable of detecting any drug-induced human antibodies sensitively (DARRTS Ref: 4366058, 12/19/2018)].

- For the dasiglucagon ADA confirmatory assay, the samples were analyzed in presence and in absence of excess dasiglucagon (5µg/mL).
 - Confirmatory Cut-Point (CCP) = initially determined to be 0.5110940 (using 0.1% confidence level false-positive rate (FPR)) during validation. A new CCP was later determined to 0.612478 using pre-dose, in-study serum samples from 30 T1DM subjects at 1% FPR (Amended study reports 18-077, (b)(4)/115) and study 14-173 ((b)(4)/037); DARRTS Ref: 4366058, 12/19/2018).
 - Assay sensitivity=13.6ng/mL (See note to Reviewer's [comment](#))
 - Low positive control (LPC) = 22.8; High positive control (HPC) = 58.2ng/mL
 - Drug tolerance of 4000ng/mL of ZP4207 for anti-ZP4207 down to 250 ng/mL
 - No Prozone up to 100mg/mL, stability at -80°C for 35 days.

[Reviewer Note: Zealand used human IgG detection controls at 60, 100 and 5000 ng/mL and human IgM detection controls at 300, 500 and 5000 ng/mL for all studies to demonstrate the assay suitability (DARRTS Ref: 4366058, 12/19/2018). For assay validation, additional sensitivity control samples were prepared at 25, 30, 35 and 40 ng/mL of the PC antibody and analyzed in the presence and absence of the study drug (5 and or 10µg/mL). The inter- and intra-assay precision data met acceptance criteria (Study number: 18-077). The NF determined using pre-dose clinical samples were used to determine plate-specific cut-point for anti-ZP4207 ADA assay (DARRTS Ref: 4366058, 12/19/2018)].

4.1.2 Anti-Glucagon antibody binding (BAb) assay:

- For anti-glucagon ADA screening assay, the plate wells (96-well) were coated with GlucaGen.
 - Positive control antibody = same as used for anti-ZP4207 BAb assay (Mab3118-M-F1-4, Lot No. MAb3118-M-F1-4-t3)
 - Assay method= ELISA
 - Secondary detection reagent: anti-mouse IgG HRP conjugate.

³ Clinical trial ZP4207-16136 is a Phase 3, randomized, double-blind, parallel group safety trial to evaluate the immunogenicity of dasiglucagon compared to GlucaGen® administered subcutaneously in patients (age between 18 and 70 years) with type 1 diabetes mellitus (DARRTS Ref: 4218432, 2/9/2018).

- Screening Cut-Point (SCP): Serum samples from 40 healthy subjects were used to determine a normalization factor, NF = 0.07734 (validation study number: 14-174 or (b) (4)/036) during assay validation.

[Reviewer Note: Zealand subsequently used pre-dose, in-study serum samples of 54 T1DM subjects from pivotal immunogenicity trial ZP4207-16136 to determine a NF of 0.05622 and to calculate PSCP for this assay and to use for subsequent clinical batches (Ref: Study number-: 18-076 (b) (4) 114), partial validation report and DARRTS Ref: 4366058, 12/19/2018)].

- For confirmatory assay, the samples were analyzed in presence and in absence of excess glucagon (5µg/mL).
 - CCP = initially determined to be 0.767265 (using 0.1% confidence level). A new CCP of 0.821605 was determined later using pre-dose serum samples from 54 T1DM subjects at 1% FPR and used in successive studies (Amended reports in study number: 18-076 (b) (4) 114) and study number: 14-174 (b) (4) 036); DARRTS Ref: 4366058, 12/19/2018).
 - Assay sensitivity=11.8ng/mL (* Reviewer's [comment](#) below)
 - LPC=22.3; HPC = 117ng/mL
 - Drug tolerance of 4000ng/mL of Glucagon for anti-Glucagon down to 250 ng/mL
 - No Prozone up to 100mg/mL, stability at -80°C for 41 days.

Table 1: Positive control antibody

Product	Mab3118-M-F1-4
Description	Monoclonal antibody to dasiglucagon
Preparation	Protein A purified
Positive control	Used in anti-dasiglucagon and anti-glucagon antibody screening, confirmatory and titer ADA assays
Epitope specificity	N-terminal part of dasiglucagon

Reviewer comment: During IND development (prior to NDA submission) OBP became aware that the sponsor used anti-mouse secondary antibodies to detect drug-induced human antibodies by antibody screening assays. The sensitivity of anti-mouse IgG towards human antibodies was estimated to be 7.2-fold less than against mouse antibodies (DARRTS Reference ID: 4221652; 02/14/2018). The Immunogenicity review team was initially concerned that anti-mouse Ig secondary antibodies may not be able to sensitively detect all drug-induced human antibodies due to species-specific affinity differences. Data showing that the anti-mouse detection reagent is able to sensitively detect all human antibodies were requested. Zealand's data showed that anti-mouse secondary reagent is able to sensitively detect human IgG subtypes and IgM (DARRTS Ref: 4221652, 02/14/2018; 4366058, 12/19/2018 and Studies 18-056, 18-076 and 18-077). FDA also recommended that the sponsor use additional sensitivity controls in each clinical sample assay plate with at least three dilutions of each human antibody isotype (IgG and IgM) with a concentration range bracketing the estimated sensitivity for these

antibodies in all ADA assays. The sponsor complied with the request and used human Ig sensitivity controls in all ADA assays.

During the course of reviewing the ADA validation studies submitted to this NDA, OBP reviewer recognized some information requested by previous reviewer which remained unaddressed by the sponsor. A request was sent to address these issues along with the NDA application (DARRTS entry ID: 4533848, 12/13/2019). The sponsor satisfactorily addressed those issues ([Appendix 1 and 2](#)).

4.2. Cross-reactivity and ADA titering assay:

All samples confirmed ADA+ for anti-ZP4207 antibody are to be tested for ADA titer, and for cross-reactivity against native glucagon using the GlucaGen ADA assay (Ref: § 5. ADA testing, below).

The titer is defined as the dilution factor of the HPC control at which the mean assay response equals the CP. The titer assay validation was reviewed by OBP and the validation was accepted (DARRTS Ref: 4332386, dated 10/10/2018). Zealand used 3 methods where the response (Y-axis) was plotted against the dilution factor (X-axis) to assess titer, supported by precision studies from 2-fold serial dilution, 4-fold serial dilution and a 5-parameter Marquardt regression analysis. Two data points, one above CP and one below CP were used to calculate the concentration of antibodies using linear regression. All three methods provided similar mean titer values. In clinical sample analysis, a confirmed ADA+ sample will be analyzed to determine the dilution that produces an assay response equal to the plate specific CP.

4.3 Neutralizing antibody (NAb) assays:

4.3.1 Anti-ZP4207 neutralizing antibody assay:

In this assay, Glucagon receptor (GR)-transfected human embryonic kidney cells (HEK GluR) were stimulated with ZP4207 in human serum, followed by measurement of the ability of ZP4207 to induce cAMP. The cAMP content was measured using an ECL assay (b) (4) (b) (4) for this assay. The validation of this assay has been reviewed, refer to DARRTS entry: 4592348, 04/15/2020 and 4669171, 09/10/2020.

- Positive control (PC): Rabbit monoclonal anti-glucagon antibody (neutralizing).
- Negative Control (NC): Pooled Human Serum (PHS) + ZP4207 (37pM)
- Cut point: $0.967 + (2.33 * 0.0804) = 1.15$; Floating cut point = $1.15 \times \text{Mean NC RLU}$.
- Assay sensitivity: 46.2ng/mL
- Drug tolerance: 1.85pM
- Assay precision – demonstrated.

4.3.2 Anti-glucagon neutralizing antibody assay:

The same assay method was used for anti-glucagon NAb assay. HEK GluR cells were stimulated with glucagon in human serum, followed by measurement of the ability of glucagon to induce cAMP. The cAMP content was measured using an ECL assay (b) (4) for this assay. The validation of this assay has been reviewed, refer to DARRTS entry 4592348, dated 04/15/2020 and 4669171, 09/10/2020.

- Positive control: Same, rabbit monoclonal anti-glucagon antibody (neutralizing).
- Negative Control (NC): Pooled Human Serum (PHS) + glucagon (73pM)
- Cut point: $0.968 + (2.33 * 0.0751) = 1.14$; Floating cut point = $1.14 \times \text{Mean NC RLU}$.
- Assay sensitivity- 45.5ng/mL
- Drug tolerance= 3.65pM
- Assay precision – demonstrated.

Reviewer Comment: Zealand recently submitted the validation reports for the anti-ZP4207 NAb assay and anti-glucagon NAb assay under IND (b) (4) along with a new anti-dasiglucagon antibody binding assay (letter dated January 20, 2020). The new anti-dasiglucagon antibody binding assay was NOT used for studies supporting this NDA; however, the anti-dasiglucagon and anti-glucagon NAb assays were used for studies under NDA 214231 (b) (4). We reviewed the assay validation reports and requested for additional information on April 14, 2020 (DARRTS Ref: 4592348, 04/15/2020, and DARRTS Ref: 45920350). The assessment of the responses to our concerns indicates that these NAb assays are suitable for use in the analysis of clinical samples from the dasiglucagon and glucagon-treated subjects from all dasiglucagon and glucagon treated studies supporting this application (Ref: DARRTS entry number- 4669171, 09/10/2020).

Zealand used commercial rabbit monoclonal anti-human glucagon IgG antibody (also known as, EP3070 and ab92517), as the positive control for both anti-dasiglucagon and anti-glucagon NAb assay. This antibody is specific for the N-terminal glucagon sequence, which is conserved between two peptides; therefore, this antibody is able to react and inhibit both glucagon and dasiglucagon responses.

The drug tolerance results for the NAb assays initially indicated that the assay performance might potentially be affected in the presence of circulating drug levels that may accumulate, particularly in samples from a multi-dose trial (b) (4). In response to our concern, the sponsor submitted additional data, which are reviewed (b) (4) and determined to be acceptable (Ref: DARRTS entry number- 4669171, 09/10/2020). Moreover, the half-life of dasiglucagon drug is 30 minutes, therefore, for a single dose study, it is unlikely that the immunogenicity sample will contain any free drug, given that these samples are normally collected after 3-4 weeks of the dose for anti-drug antibody evaluation. Therefore, the drug tolerance is unlikely be an issue for these NAb assays during the analysis of clinical samples from single-dose studies under NDA 214231.

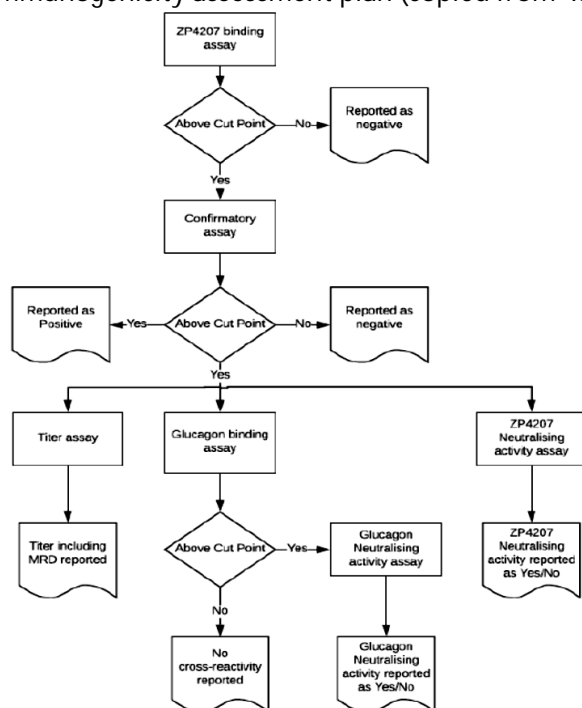
Overall, the immunogenicity assays used to monitor the immune response are appropriately validated per FDA Guidance, *Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection (2019)*.

5. ADA Testing Strategy:

A tiered antibody assay approach was used to monitor the development of ADA, as recommended by the Agency. A summary of the strategy is presented below with a graphical overview of the strategy copied from the submission.

- In Tier 1, screening for anti-dasiglucagon antibodies (ADA) was carried out in subjects treated with dasiglucagon followed by confirmatory analysis in Tier 2.
- Two ADA binding assays were used in parallel, in Tier 1: anti-dasiglucagon antibody binding assay was used to detect antibodies to dasiglucagon in dasiglucagon-treated subjects and the anti-glucagon antibody binding assay was used to detect antibodies to glucagon in glucagon-treated subjects.
- In Tier 3, confirmed ADA+ samples were subjected to analyze for their ability to neutralize the effect of either dasiglucagon or glucagon in a cell-based assay.
- The dasiglucagon ADA+ samples were further characterized in the anti-glucagon antibody analyses in order to assess for their ability to cross-react with glucagon.
- Titration was performed in confirmed ADA-positive samples.

Figure 2: Immunogenicity assessment plan (copied from 'ISI original Figure 4-1)':



MRD: Minimum required dilution; ZP4207: dasiglucagon

6. Overview of Clinical Immunogenicity Trials:

The development of anti-drug antibodies were evaluated in samples from 9 clinical trials including a phase 3 pivotal immunogenicity trial (Table 2) and two supportive multi-dose clinical trials



A total of 498 subjects were exposed to dasiglucagon (370 adult patients, 20 pediatric patients and 108 healthy subjects) per the Integrated Summary of Immunogenicity [5.3.5.3]. Clinical studies supporting this application are described in Table 2 below, copied from the 'Integrated Summary of Immunogenicity'.

Table 2: Dasiglucagon clinical trials for severe hypoglycemia

Indication	Phase 1/Clinical pharmacology trials	Phase 2 trials	Phase 3 trials
Treatment of severe hypoglycemia	Healthy volunteers	Patients with T1DM	Patients with T1DM
	14013, Part 1 – First in man trial	15126 – Dose-finding trial	16137 – 1 st pivotal trial
	15007 – Multiple ascending dose trial		17145 – 2 nd pivotal trial
	17144 – IV/QTc trial		17084 – Bridging trial
Dual-hormone artificial pancreas (DHAP)	Patients with T1DM	Patients with T1DM	16136 – Immunogenicity trial
	14013, Part 2 – First in man trial		17086 – Pediatric trial
		16098 – Low-dose range trial	
		16051 – Pump feasibility trial	

Abbreviations: T1DM: type 1 diabetes.

7. ADA sampling time for the clinical trials:

The ADA sampling time for the clinical trials were designed with follow up periods for at least 3-4 weeks after dosing in order to adequately assess immunogenicity. Additional follow-up visits were performed in order to follow the ADA+ patients until they returned to baseline. The sponsor collected all samples on dosing days before dosing. An overview of the antibody collection time points are presented in the following Table 3 (revised by the reviewer for brevity from the original table 5-3 submitted in the Integrated Summary of Immunogenicity).

Table 3: ADA sampling time points per clinical trial

Trial		Planned Visits*						Additional FU visit (ADA+)		
		Pre-dose	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56		
Phase-I										
14013	Part-I	x	x			x(FU)				
	Part-II	x	x	x						
15007		x				x(FU)				
17144		x				x(FU)				

Phase-II										
15126		X	X			X(FU)			No FU sampling	
16051	(b) (4)	X		X		X(FU)				
16098		X			X		X(FU)		3.5 months	7 months
Phase-III										
16136	Immuno-genicity	X	X	X			X(FU day 35)	X (FU days 60 & 104)		
16137	1 st pivotal	X				X(FU)			14 months	17 months
17145	2 nd pivotal	X				X(FU)				
17086	Pediatric pivotal	X				X(FU)			12 months	19 months (June 2020)
17084	bridging	X				X		X(FU)		

* Actual visit window may vary between studies

Reviewer comment: The sampling time for testing the development of anti-drug antibodies is adequately designed as determined by the immunogenicity reviewer at various occasions (DARRTS Reference ID: 4153596, 09/15/2017; Reference ID: 4215993, 2/2/2018; Reference ID: 4218432, 02/09/2018).

8. Analysis of immunogenicity data – summary of clinical data:

To analyze the clinical samples the sponsor determined new plate-specific cut-point (PSCP) using pre-dose serum samples from trial subjects in each study (DARRTS Reference ID: 4221652, 02/14/2018). The appropriate t-test was carried out to determine if there was any statistically significant difference between the population used in the validation study and the patients from the first batch in the clinical trial. The new PSCP was used to evaluate clinical samples, to identify patients with treatment induced or treatment boosted ADA response. Human IgG and IgM were used as sensitivity control in all assay runs. The sponsor demonstrated assay precision before analyzing the clinical samples from all studies.

For confirmatory assay, the sponsor initially used 0.1% confidence interval to analyze clinical samples from studies [ZP4207-14013](#), [ZP4207-15007](#) ([\(b\) \(4\)](#) 048), [ZP4207-15126](#), [ZP4207-16051](#), [ZP4207-16098](#) and [ZP4207-16136](#), later these samples were re-evaluated with a 1% interval based on the recommendation provided in the FDA immunogenicity guidelines⁴. One patient from study ZP4207-15126, became ADA+ as a result of the re-evaluation of samples using new CCP. However, the confirmatory cut-point assessment results using a new cut-point based on 1% FPR could not be found for any of these assays. A request was communicated for submitting those data (DARRTS Reference ID: 4683824, 10/09/2020). The sponsor satisfactorily responded to our information request (IR) on 10/16/2020 (see Zealand response below, [Appendix 3](#)). ADA

⁴ Immunogenicity Testing of Therapeutic Protein Products - Developing and Validating Assays for Anti-Drug Antibody Detection, January 2019; <https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.

incidence results are summarized in Table 4, below, revised by the reviewer from information presented in ISI Table 6-1.

Table 4: List of trials and immunogenicity results

Trial ID	Treatment duration/doses	No. of subjects exposed to dasiglucagon	No. of anti-dasi ADA+ subjects	No. of subjects exposed to Glucagon	No. of anti-Gluc Ab+ subjects	Follow up
14013	SD Dasiglucagon + SD Glucagon	HV: 48	0	16	0	28 days from 1 st dose
		T1DM: 20		20	0	14 days from 1 st dose
15007	5 doses on consecutive days	HV: 18	0		n/a	23 days
15126 Gr1 parallel Gr2-4 cross-over	SD Dasiglucagon + SD Glucagon	T1DM: 55	1	34	0	3 weeks
16051 (b) (6) cross-over	SD Dasiglucagon + SD Glucagon	T1DM: 10	0	12	0	2-3 weeks after last dose
16098 (b) (6) cross-over	4 dosing visits (2 doses/visit), 3-7 days between dosing visits	T1DM: 22	1	23	1	3 weeks after last dose
16136 Immunogenicity	3 SD 1 week apart	T1DM: 57	0	54	0	15 weeks from 1 st dose
16137	SD	T1DM: 82	1	43	0	4 weeks
17084	2 SD Dasiglucagon, different batches, 4 weeks between doses	T1DM: 90	0	n/a	n/a	8 weeks from 1 st dose
17086 Pediatric pivotal	SD	T1DM: 20	1	10	0	4 weeks
17144	SD	HV: 42	0	n/a	n/a	4 weeks
17145	SD	T1DM: 32	0		n/a	4 weeks
Total		498	4	212	1	

SD: Single dose, (b) (4)

[Reviewer Notes:

1. Studies 16051, 16098 and 17084 were cross-over studies, therefore reported immunogenicity cannot be associated with specific treatment (dasiglucagon or glucagon).
2. Studies 16051 and 16098 were submitted as supportive data for immunogenicity. (b) (4)

A total of 4 of the 498 patients (0.8%) who received dasiglucagon during the clinical trials supporting the NDA had treatment-induced anti-drug antibody formation. On the other hand, one of 212 patients (0.5%) developed antibodies to glucagon. None of the patients in the phase 3 pivotal immunogenicity trial 16136 who received 3 weekly doses of dasiglucagon, glucagon or placebo, developed antibodies to dasiglucagon or glucagon (Table 4) by 4 weeks of follow-up.

8.1 ADA assay results from individual trials:

a. Study: ZP4207-14013 (Phase 1 FIH trial, (b) (4) study number (b) (4)/033)

This study had two parts. Part I included dose escalation of ZP4207 in 8 cohorts of healthy individuals (HV), each with 8 subjects, randomized at 3:1 to receive either a single ascending dose (0.01, 0.1, 0.3, 1.0, and 2.0 mg SC and 0.3, 1 and 2 mg IM using vial and syringe) or a single fixed dose (1.0 mg) of ZP4207. The samples were collected at pre-dose, 7±3 days post-dose (Visit 3), and 28±3 days post-dose (Visit 4) for testing anti-ZP4207 and anti-glucagon antibodies.

In Part II, group of 10 insulin-induced hypoglycemic T1DM subjects were treated with fixed single dose of ZP4207 (0.7 mg) and glucagon (1.0 mg) in a sequential cross-over design in randomized order. Blood samples were collected before dose at dosing Visit 2 and 3 (1 week apart) and 7±3 days post second dose (Visit 4) for ADA test.

Results - Part I: Of 160 samples, 52 were initially screened as positive for anti-ZP4207 antibodies, while one of these 52 samples was confirmed positive for ADA with a titer of 1.14 (False positive rate, FPR = 51/160, 31.9%; Tables 5a and 5b). This ADA+ sample was a pre-dose sample from subject no. (b) (6) (Ref: Protocol : ZP4207-14013; Appendix 16.2.6.1.2 Immunogenicity Data, Page 80 of 1573). Hence, this subject was considered as base-line ADA+, rather than treatment-induced. The same sample was tested negative by anti-glucagon antibody assay.

Ninety-six (96) samples were tested for anti-glucagon antibodies, 11 samples were screened positive for anti-glucagon antibodies, but none were confirmed ADA+ (FPR=11/96, 11.5%).

Table 5a: ADA results for anti-ZP4207 in samples from study ZP4207-14013 (Part I)

ZP4207-14013 Part 1	1013	VISIT 2	Pre-Dose		Anti-ZP4207 Screening test	Positive	SERUM	ELISA	(b) (4)
ZP4207-14013 Part 1	1013	VISIT 2	Pre-Dose		Anti-ZP4207 Confirmatory Test	Positive	SERUM	ELISA	(b) (4)
ZP4207-14013 Part 1	1013	VISIT 2	Pre-Dose		Anti-ZP4207 titre	1.14	SERUM	ELISA	(b) (4)
ZP4207-14013 Part 1	1013	VISIT 2	Pre-Dose		Anti-Glucagon Screening test	Negative	SERUM	ELISA	(b) (4)

Table 5b: Titer of confirmed ADA+ samples (anti-ZP4207) from study ZP4207-14013 (Part I)

Subject	Timepoint	Dilution factor	Mean absorbance	%CV	Plate specific CP	Result	Titre	Start Date of Analysis	Run ID
(b) (6)	Part1/Visit 2	1	0.13185	10.4	0.12505*	Positive	1.14	(b) (6)	HuS28R Titre ZP4207
		2	0.08240	6.0		Negative			
		4	0.05010	4.5		Negative			
		8	0.03600	4.3		Negative			

*Titre calculated using the NF which was recalculated using the Part1/Visit 2 samples. The validated NF was used to assess the performance of the run.

Results - Part II: Of 60 samples, 8 were screened ADA+ based on the PSCP from pre-dose study samples (Table 6). Three of 8 putative positive samples were confirmed ADA+ for the presence of anti-ZP4207 antibodies (FPR = 5/60, 8.3%). All 3 ADA+ samples were from subject (b) (6) (Table 7). The titer for these samples were determined to be 1.62, 2.20 and 1.52 respectively for samples at visit 2, visit 3 and FU visit (Table 8 below). The pre-dose sample was tested negative by anti-glucagon antibody screening assay.

Fifteen of 60 samples were screened positive for anti-glucagon antibodies, but none were confirmed ADA+ for the anti-glucagon antibodies (FPR=15/60, 25%) (Appendix 16.1.13 Bioanalytical reports, Study number (b)(4) 040).

Table 6: ADA results for anti-ZP4207 in samples from study ZP4207-14013 (Part II)

ZP4207-14013 Part 2	(b)(6)	VISIT 2	Pre-Dose		Anti-ZP4207 Screening test	Positive	SERUM		ELISA	(b)(6)
ZP4207-14013 Part 2	(b)(6)	VISIT 2	Pre-Dose		Anti-ZP4207 Confirmatory Test	Positive	SERUM		ELISA	(b)(6)
ZP4207-14013 Part 2	(b)(6)	VISIT 2	Pre-Dose		Anti-ZP4207 titre	1.62	SERUM		ELISA	(b)(6)
ZP4207-14013 Part 2	(b)(6)	VISIT 2	Pre-Dose		Anti-Glucagon Screening test	Negative	SERUM		ELISA	(b)(6)

Table 7: ADA results for anti-ZP4207 in samples from study ZP4207-14013

Subject	Timepoint	Mean absorbance with ZP4207	Mean absorbance without ZP4207	Ratio	Plate specific CP	Result	Date of Analysis	Run ID
(b)(6)	Part 2/ Visit 4	0.08075	0.10220	0.7901	0.29214	Negative	(b)(6)	HuS29 Con ZP4207
(b)(6)	Part 2/Visit 2	0.04480	0.13315	0.3365	0.29214	Positive	(b)(6)	HuS29 Con ZP4207
(b)(6)	Part 2/Visit 3	0.04960	0.11040	0.4493	0.29214	Positive	(b)(6)	HuS29 Con ZP4207
(b)(6)	Part 2/ Visit 4	0.05055	0.11915	0.4243	0.29214	Positive	(b)(6)	HuS29 Con ZP4207
(b)(6)	Part 2/ Visit 4	0.09590	0.10340	0.9275	0.29214	Negative	(b)(6)	HuS29 Con ZP4207
(b)(6)	Part 2/ Visit 2	0.15405	0.16610	0.9275	0.27140	Negative	(b)(6)	HuS32 Con ZP4207
(b)(6)	Part 2/Visit 3	0.15690	0.17500	0.8966	0.31526	Negative	(b)(6)	HuS37 Con ZP4207
(b)(6)	Part 2/ Visit 4	0.17240	0.17330	0.9948	0.31526	Negative	(b)(6)	HuS37 Con ZP4207

Confirmatory CP = 0.51109

[Reviewer Note: The footnote in table 7 indicates that the CCP for dasiglucagon used in analyzing the ADA results, was determined during assay validation study using 0.1% FPR instead of using 1% FPR as recommended for CCP determination. In response to FDA IR comment on 10/09/2020, the sponsor confirmed that they used the revised CCP of 0.612478 for dasiglucagon determined using 1% FPR and re-evaluated the results manually but did not update the footnote in table 7 (see § 8 Analysis of immunogenicity data – summary of clinical data). The results in table 7 did not change as a result of using the revised CCP of 0.612478 for anti-dasiglucagon confirmatory assay.]

Table 8: Titer determination of confirmed ADA+ samples (anti-ZP4207) from study ZP4207-14013 (Part2)

Subject	Timepoint	Dilution factor	Mean absorbance	%CV	Plate specific CP	Result	Titre	Start Date of Analysis	Run ID	
(b)(6)	Part2/Visit 2	1	0.19060	1.0	0.14218*	Positive	1.62	(b)(6)	HuS31 Titre ZP4207	
		2	0.11260	1.9		Negative				
		4	0.07420	3.8		Negative				
		8	0.04700	1.5		Negative				
	Part2/Visit 3	1	0.17280	3.0	0.10906*	Positive	2.20			HuS34 Titre ZP4207
		2	0.11350	0.7		Positive				
		4	0.06890	5.5		Negative				
		8	0.04815	2.8		Negative				
	Part2/Visit 4	1	0.16345	6.4	0.14218*	Positive	1.52		HuS31 Titre ZP4207	
		2	0.12230	6.7		Negative				
		4	0.07680	2.8		Negative				
		8	0.05080	8.1		Negative				

*Titre calculated using the NF which was recalculated using the Part1/Visit 2 samples. The validated NF was used to assess the performance of the run.

Reviewer comment: The subject no. (b) (6) from part I of this study was confirmed ADA+ for the presence of anti-dasiglucagon antibodies at visit 2 (pre-dose) sample with a titer of 1.14 (at MRD of 1:10). This ADA+ sample was tested negative at further dilution in titer analysis (Table 5b) indicating a weaker antibody. This subject was considered of having pre-existing antibodies to dasiglucagon.

Three samples from subject (b) (6) of part II of this study (at Visit 2, Visit 3 and FU), were confirmed ADA+ for the presence of anti-ZP4207 antibodies. The titer for these samples were determined (Table 8), which did not change significantly for Visit 3 and FU samples in comparison to pre-dose antibody titer (Table 8). Therefore, none were considered treatment-emergent ADA+.

The false positive rate for the anti-dasiglucagon antibody screening assay for the Part I (FPR = 31.9%) and anti-glucagon antibody assay for the Part II (FPR = 25%) of the trial were high. In general, the FPR is expected to vary between 2-11%⁵ for the screening assay. Although the reason for the high FPR is unclear, the assay was run in presence of additional human IgG and IgM assay sensitivity controls as suggested by FDA and the assay precision demonstrated the reliability of the assay. Initial screened positive samples are subsequently assessed in the confirmatory assay to determine true-positives. Therefore, high FPR did not increase the risk of not identifying a true positive sample by confirmatory assay, and did not have a negative impact on the study results. Additionally, the overall FPR from all studies under this NDA was lower and acceptable for both ADA screening assays (Table 17). Therefore, the screening assay results are acceptable.

b. Study: ZP4207-15007 (Phase1b-MAD, (b) (4) study number (b) (4)/048)

In this placebo-controlled phase 1b study, five repeat doses of dasiglucagon (at 0.1, 0.3 and 1.0 mg) were administered subcutaneously on 5 subsequent dosing days in 24 healthy volunteers. The presence of anti-ZP4207 antibodies was investigated in 48 samples collected at pre-dose and 28 days post-dose (FU).

Results: None of the samples had an absorbance that was above PSCP, so no confirmatory assay was run for these samples. Individual immunogenicity data are presented by subject in Listing 16.2.13 which is not copied here (Page 1135 – 1139; study number (b) (4)/048).

[Reviewer Note: A new NC was established from pre-dose serum samples of 24 individual from this study ((b) (4) 048), box-plot did not identify any outlier. However, pre-dose serum samples (2 ml each) from subjects (b) (6) were pooled to establish NC (data presented, not copied here). The CP was determined using 24 pre-dose samples after eliminating outliers by applying box-plot. The results were

⁵ Goodman, Cowen, Devanarayan et al. (2018): Feedback from the European Bioanalysis Forum: focus workshop on current analysis of immunogenicity: best practices and regulatory hurdles. Conference report, Bioanalysis Vol 10, No 4, 197-204.

tested for normal distribution and accepted for normal distribution, therefore CP was determined using the mean of the remaining values +1.645 times the standard deviation.

$$\begin{aligned} \text{CP} &= 0.07096 + (1.645 \times 0.02781) = 0.11670745 \\ \text{Global mean NC} &= 0.06098333333; \text{NF} = 0.05572411667 \\ \text{Plate specific CP} &= \text{mean assay NC} + 0.05572411667 \end{aligned}$$

Reviewer comment: The new NC was established demonstrating that the NC samples from older study and the new NC samples are not significantly different. The sponsor provided data which I reviewed and determined to be acceptable (pivotal immunogenicity trial ZP4207-16136).

The sponsor stated that none of the 48 samples collected from this study had an absorbance that was above PSCP, so no confirmatory assay was run for the samples from this study. In order to ensure that the determination of the assay CP was appropriate, the data related to the cut-point determination was reviewed and determined to be appropriate. Pre-dose clinical samples from 24 subjects were used to determine the PSCP, no outlier found. The normalization factor (NF) was then determined from the mean absorbance data obtained from the pre-dose study samples. The assay precision met the acceptance criteria. Therefore, the ADA test results are acceptable.

c. Study: ZP4207-17144 (Phase 1, (b) (4) study Number (b) (4) /119)

This was a dose-escalation trial to evaluate the safety and tolerability of a *single IV* dose of dasiglucagon and the bioavailability of dasiglucagon following SC administration compared to IV administration in healthy subjects. In this single dose trial (60 HV), antibodies against dasiglucagon, were measured at the dosing visit (visit 2) and at FU (visit 3). Twenty-two pre-dose samples were used to determine plate-specific cut-point.

Results: Of 84 samples tested, 13 samples were screened ADA+ (Table 9a), and only 2 of 13 samples (pre-dose and FU samples) from one subject (ID # (b) (6)), were confirmed ADA+ for the presence of anti-dasiglucagon antibodies (FPR=11/84, 13.1%). The titer for these ADA+ samples (at pre-dose and at FU sample) were determined to be 32.1 and 34.9 respectively that indicates that the titer results for the FU sample was not significantly different compared to pre-dose (less than 4-fold increased; at least 4-fold titer increase is required to be considered as treatment-emergent ADA-positive according to the protocol); therefore, the response was not classified as treatment-boosted (Table 9b). The potential cross-reactivity of this sample with glucagon was tested, however, no cross-reactivity to endogenous glucagon was found.

Table 9a: Confirmatory assay results for anti-ZP4207 in samples from clinical trial ZP4207-17144

Subject	Visit	Mean absorbance with ZP4207	Mean absorbance without ZP4207	Ratio	Plate Specific Cut Point	Result	Date of Analysis	Run ID
(b) (6)	3	0.14360	0.14740	0.974	0.20034	NEGATIVE	(b) (6)	HuS04 CON
	3	0.12700	0.11670	1.09	0.18431	NEGATIVE		HuS05 CON
	2	0.16010	0.38480	0.416	0.20034	POSITIVE		HuS04 CON
	3	0.16820	0.40310	0.417	0.20034	POSITIVE		HuS04 CON
	2	0.20870	0.22570	0.925	0.20034	NEGATIVE		HuS04 CON
	3	0.21205	0.22700	0.934	0.20034	NEGATIVE		HuS04 CON
	2	0.20490	0.17130	1.20	0.20034	NEGATIVE		HuS04 CON
	2	0.15865	0.19210	0.826	0.20034	NEGATIVE		HuS04 CON
	3	0.19790	0.20510	0.965	0.20034	NEGATIVE		HuS04 CON
	2	0.18135	0.19490	0.930	0.20034	NEGATIVE		HuS04 CON
	3	0.22330	0.21445	1.04	0.20034	NEGATIVE		HuS04 CON
	2	0.29320	0.28950	1.01	0.20034	NEGATIVE		HuS04 CON
	3	0.30275	0.28930	1.05	0.16272	NEGATIVE		HuS06 CON

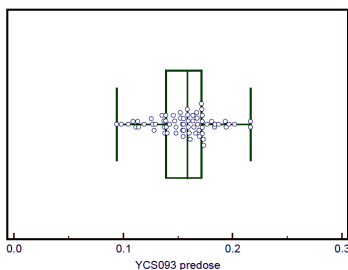
Confirmatory CP = 0.612478

Table 9b: Titer of confirmed ADA-positive samples from clinical trial ZP4207-17144

Subject	Visit	Dilution Factor	Mean Absorbance	%CV	Plate Specific Cut Point	Result	Titre (Titre x MRD)	Date of Analysis	Run ID
(b) (6)	2	1	0.35925	2.5	0.18120	POSITIVE	3.21 (32.1)	(b) (6)	HuS07 TITRE
		2	0.24870	2.0		POSITIVE			
		4	0.13715	2.0		NEGATIVE			
		8	0.07780	1.8		NEGATIVE			
		16	0.04285	5.1		NEGATIVE			
		32	0.02300	8.0		NEGATIVE			
		64	0.01570	6.3		NEGATIVE			
		128	0.00980	13.0		NEGATIVE			
	3	1	0.35265	1.0	0.18120	POSITIVE	3.49 (34.9)		HuS07 TITRE
		2	0.27315	2.9		POSITIVE			
		4	0.14945	3.7		NEGATIVE			
		8	0.08970	2.4		NEGATIVE			
		16	0.04715	2.2		NEGATIVE			
		32	0.02595	11.7		NEGATIVE			
64	0.01710	8.3	NEGATIVE						
128	0.01575	46.2	NEGATIVE						

MRD = Method requires a 10 fold dilution prior to analysis

[Reviewer Note: A new NC sample was established for this assessment using pre-dose samples from clinical trial ZP4207-16137 (b) (4)/093). The absorbance data collected from each pre-dose sample in the screening assay in (b) (4)/093 was analyzed using a Box Plot to identify outliers, which were removed from the data set. This was repeated until no further outliers was identified (see the box-plot below). The entire volume of each pre-dose sample which remained in the data set after outlier removal was pooled to create new NC.



This Box Plot was copied from the submission showing the establishment of NC using pre-dose samples from trial ZP4207-16137 (b) (4)/093) – after removal of all outliers. The new cut point

and NF were calculated based on the Visit 2 pre-dose samples (data submitted). NF for screening assay = 0.05346; PSCP (floating CP) = mean NC + 0.05346; CCP = 0.612478, was determined using 1% CL.]

Reviewer Comment: A new negative control (NC) was established since the existing NC ran out. In order to establish a new NC, a demonstration that the NC samples from older study and the new NC are not significantly different is needed. The sponsor provided data comparing the NC from previous study (pivotal immunogenicity trial ZP4207-16136, (b) (4)/081), which are not copied here but reviewed to be acceptable. A new NF and the new cut-point were then determined to analyze samples from this study based on the absorbance data of Visit 2 pre-dose samples. This approach is appropriate to establish a new NC, therefore, acceptable.

None of the study subjects were found to have treatment-induced ADA+ samples in this study, however, one subject had pre-existing antibodies to dasiglucagon.

d. Study: ZP4207-15126 (Phase 2, (b) (4) study number (b) (4)/065)

This was a Phase 2 dose-finding study conducted in subjects with T1DM (of 4 cohorts). One cohort of 8 patients (6 and 2 patients received single dose of ZP4207 and GlucaGen respectively in a parallel set-up) and 3 cohorts of 16 patients (each patient received a single dose of ZP4207 and Glucagon in a crossover setup) were planned. The dasiglucagon doses of 0.1, 0.3, 0.6 and 1.0mg were administered subcutaneously. The presence of ADA to ZP4207 or glucagon was tested at pre-dose (V2), Visit 3 (pre-dose for Group 2-4), and at Visit 4 (FU) for all groups.

Results: Of 166 samples (from 58 T1DM subjects), 13 samples (3 of the 18 samples from Group 1; 3 of 50 samples from Group 2; 1 of 50 samples from Group 3 and 6 of 48 samples from Group 4) were screened positive for the presence of anti-ZP4207 antibodies (data not copied here). One of these 13 putative ADA+ samples was confirmed ADA+ by the confirmatory assay (FPR=12/166, 7.2%). This sample was initially recognized by the sponsor to be ADA-negative by using the CCP which was determined during the validation of this assay at 0.1% FPR (footnote of Table 10a). Later the sponsor reassessed all samples using a new CCP established using 1% FPR (CCP = 0.612478). As a result, the visit 2 pre-dose sample from subject # (b) (6) turned to be ADA+ (Table 10a). However, the footnote for CCP in Table 10a was not changed. In response to FDA comment for clarification on 10/09/2020, the sponsor stated that they reevaluated the results using the new CCP and manually determined that this sample was ADA+ but did not update the table with the revised new ADA information. The antibody titer for this sample was determined to be 22 (ISI report, page 61). This sample was tested by anti-glucagon antibody assay and found negative to cross-react with glucagon.

The sponsor stated that this subject (b) (6), experienced a mild, temporary event of hypoglycemia approximately 6 hours after administration of 0.5mg of glucagon (5 days after first dosing with dasiglucagon), and tested anti-dasiglucagon antibody positive at the glucagon dosing day with a titer of 22. The sample was tested non-crossreactive with glucagon, so this event of hypoglycemia may not be ascribed to ADA formation.

Table 10a: Confirmatory assay results for anti-zp4207 in serum samples obtained from study ZP4207-15126 Group 2

Subject	Visit	Mean absorbance with ZP4207	Mean absorbance without ZP4207	Ratio	Plate specific CP	Result	Date of Analysis	Run ID
(b) (6)	2	0.2640	0.4288	0.616	0.27977	Negative	(b) (6)	HuS28 CON
	3	0.2172	0.3962	0.548	0.27977	Negative		HuS28 CON
	4	0.2333	0.4053	0.576	0.27977	Negative		HuS28 CON

Confirmatory CP = 0.51109

Eight of 154 samples were screened positive for anti-glucagon antibodies and 4 of which were tested confirmed ADA+ (FPR=4/154, 2.6%). Of these 4 ADA+ samples, 2 samples were from patient (b) (6) (pre-dose and visit 3; Group 2), one sample each from patient (b) (6) (pre-dose, Group 3) and (b) (6) (pre-dose, Group 4) were ADA+ for anti-glucagon antibodies (Table 10b).

Table 10b: Confirmatory assay results for anti-Glucagon in serum samples obtained from study ZP4207-15126 Group 2:

Subject	Visit	Mean absorbance with Glucagon	Mean absorbance without Glucagon	Ratio	Plate specific CP	Result	Date of Analysis	Run ID
(b) (6)	2	0.11410	0.15525	0.735	0.18343	Confirmed positive	(b) (6)	HuS24 CON
	3	0.08865	0.13700	0.647	0.18343	Confirmed positive		HuS24 CON
	4	0.19365	0.21390	0.905	0.18343	Negative		HuS24 CON

Confirmatory CP = 0.76727

Group 3:

(b) (6)	2	0.12440	0.17275	0.720	0.18343	Confirmed positive		HuS24 CON
	3	0.14010	0.16390	0.855	0.18343	Negative		HuS24 CON
	4	0.15320	0.17325	0.884	0.18343	Negative		HuS24 CON
	2	0.18975	0.19705	0.963	0.18343	Negative		HuS24 CON

Confirmatory CP = 0.76727

Group 4:

(b) (6)	2	0.10915	0.15915	0.686	0.18343	Confirmed positive		HuS24 CON

Confirmatory CP = 0.76727

[Reviewer Note: The footnote in table 10b for groups 2, 3 and 4 indicates that the sponsor used a CCP of 0.76727 in analyzing the clinical samples from this study, which was determined during assay validation study using 0.1% FPR. In response to FDA comment on 10/09/2020 for a clarification, the sponsor confirmed that they recalculated the CCP to 0.821605 using 1% FPR as recommended for glucagon and re-evaluated the results manually and since the final findings did not change, they did not replace the table with an updated footnote (see § 8 Analysis of immunogenicity data – summary of clinical data, and [Appendix-3](#).)]

The titer of all 4 ADA+ samples were determined (44.6, 23, 10.4 and 16.8 respectively for visits 2 and 3 of patient (b) (6) and visit 2 samples of patient (b) (6) and (b) (6)); presented in Table 10c.

Pre-dose samples from Patients (b) (6) and (b) (6) were tested negative at subsequent visits, therefore, it was considered that the response was not due to glucagon drug administration. Sample from Patient (b) (6) was ADA+ at visit 3 prior to GlucaGen administration with an ADA titer lower than the titer observed for Visit 2 sample (Table 10c) and tested negative at the FU

visit (V4), therefore, this response from Visit 3 sample was also not considered treatment-induced.

Table 10c: Titer of confirmed ADA-positive study samples (anti-glucagon)

Subject	Visit	Dilution factor	Mean absorbance	%CV	Plate specific CP	Result	Titre (Titre x MRD)	Start Date of Analysis	Run ID
(b) (6)	2	1	0.26520	5.2	0.17789	Positive	4.46 (44.6)	(b) (6)	HuS25 Titre
		2	0.20380	2.5		Positive			
		4	0.18110	3.1		Positive			
		8	0.15350	8.4		Negative			
	3	1	0.25505	4.5	0.17789	Positive	2.30 (23.0)		HuS25 Titre
		2	0.18425	7.1		Positive			
		4	0.14175	2.4		Negative			
		8	0.16105	0.3		Negative			
	2	1	0.17980	5.3	0.17789	Positive	1.04 (10.4)		HuS25 Titre
		2	0.13000	2.6		Negative			
		4	0.13140	11.0		Negative			
		8	0.13535	10.0		Negative			

MRD = Method requires a 10 fold dilution prior to analysis

Reviewer comment: No treatment-induced, or treatment-boosted anti-ZP4207 ADA or anti-glucagon antibody incidents were observed in this study. All patients treated with ZP4207 were classified as ADA-negative (Appendix 16.2.14; Individual Listing of Anti-Drug Antibodies Test Data; Page 1760).

[Reviewer Note: The sponsor established a new NC and adjusted the NF from the validation study (b) (4)/037). A new NF was calculated by using six batches (HuS15, HuS16, HuS17, HuS18, HuS26, and HuS27R) and the new NC. The NF was found to be 0.086381 (data submitted, I reviewed but did not copy here).

They compared the pre-dose sample data from all subjects with the data obtained in the validation study (b) (4)/037) and was concluded that the two means were significantly different. Therefore, the CP was recalculated from pre-dose samples (excluding outlier), which were determined to be normally distributed, so, the CP was calculated using mean of the remaining values + 1.645 times the standard deviation.

$$CP = 0.1998 + (1.645 \times 0.08018) = 0.3316961; NF = CP - \text{global mean of NC} = 0.3316961 - 0.201719 = 0.1299771$$

For, anti-glucagon antibody assay, they followed the same procedure, but when compared pre-dose sample data from all subjects with the data obtained from the validation study (b) (4)/036), the means were found to be not significantly different, therefore recalculation of CP was not necessary. The following results were used.

$$NF \text{ for screening assay} = 0.075504583; PSCP \text{ (floating CP)} = \text{mean assay NC} + 0.075504583; CCP = 0.767265]$$

Reviewer comment: The new NC was established demonstrating that the NC samples from older study and the new NC are not significantly different. The sponsor provided data comparing the NC from previous study (pivotal immunogenicity trial ZP4207-16136, (b) (4)/081), which is acceptable.

e. Study: ZP4207-16136 (Phase 3, immunogenicity, (b) (4) study number- (b) (4)/081)

This phase 3 *parallel arm* immunogenicity study protocol was reviewed earlier (DARRTS Reference ID: 4121026; 07/06/2017). A total of 57 subjects with T1DM were exposed to dasiglucagon (0.6mg by SC) and 54 subjects with T1DM were exposed to glucagon (1.0mg by SC) in 3 consecutive weekly doses in each arm.

Antibody development against dasiglucagon and glucagon was assessed in samples collected at pre-dose, Day 7 ±1 (V3), Day 14 ±1 (V4), Day 35 ±2 (V5), Day 60 ±5 (V6) and Day 104 ±10 (EoT) after first dose. The PSCP and NF were calculated based on pre-dose samples. The mean absorbance data for the study samples from this study is presented in Table 4 of the study report (b) (4)/081 which is not copied here.

Results: Seventy of the 330 samples screened positive for anti-ZP4207 antibodies, 3 of which were tested to be confirmed ADA+ by the confirmatory assay (FPR= 67/330, 20.3%). All 3 samples were from subject (b) (6) and were collected at Visit 2 (pre-dose), Visit 3 (Day 7), and Visit 7 (Day 104). The samples from subject (b) (6) were further analyzed in titering assay. The titers were determined to be 14.0, 13.3, and, 32.8 for samples at V2 (baseline), V3 (Day 7) and V7 (Day 104) respectively (Table 11a and Table 14.2.4.1.2).

A total of 315 samples were screened by anti-glucagon assay, 9 samples were screened putatively positive for anti-glucagon antibodies, but none of these samples were confirmed positive for anti-glucagon antibodies (FPR= 9/315, 2.9%).

Reviewer comment: The FPR results for anti-dasiglucagon antibody assay appears high (generally it may vary between= 2-11%⁶). The reason of higher FPR is unclear. If the in-study baseline samples yielded an FPR outside these limits, a statistical comparison of the means and variances between the validation data and the in-study pre-dose baseline data should be made to further understand the differences as per Goodman et. al., 2018⁶ and Shankar et. al., 2008⁷, and if significant difference is found, a new in-study cut-point is advised to establish. The sponsor performed this activity and determined a new plate-specific CP using pre-dose study samples to analyze clinical samples, the FPR still remains higher. Visual inspection of mean absorbance data for each of these 70 samples indicate that 25 samples (36% samples) had mean absorbance value closer to the plate-specific cut-point (within 10% of the CP). Nevertheless, high FPR did not increase the risk of not identifying a true positive sample by subsequent confirmatory assay and therefore did not expect to have negative impact on the

⁶ Goodman, Cowen, Devanarayan et al. (2018): Feedback from the European Bioanalysis Forum: focus workshop on current analysis of immunogenicity: best practices and regulatory hurdles. Conference report, Bioanalysis Vol 10, No 4, 197-204.

⁷ G. Shankar et al. / Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1267–1281

study results. Additionally, the overall FPR from all studies was within the acceptable range for both ADA screening assays (see [Table 17, below](#)).

The titer assay provides an understanding of how much antibodies are present. The greater the concentration of the specific antibody in the serum sample, the higher the titer and is determined by serially diluting the serum samples containing antibodies and evaluating the sample where the reaction is still detectable. For these samples the antibody titer was determined at dilution equivalent to MRD indicating a low titer of the antibodies.

Table 11a: Titer determination of confirmed ADA+ samples from Trial ZP4207-16136 (anti-ZP4207)

Subject	Visit	Dilution factor	Mean absorbance	%CV	Recalculated Plate specific CP*	Result	Titre (Titre x MRD)	Date of Analysis	Run ID
(b) (6)	Visit2pd	1	0.22205	4.1	0.19508	Positive	1.40 (14.0)	(b) (6)	HuS19R Titre
		2	0.15505	2.0		Negative			
		4	0.08865	5.8		Negative			
		8	0.06340	7.4		Negative			
		16	0.05895	19.1		Negative			
		32	0.05520	77.6		Negative			
		64	0.03870	42.4		Negative			
		128	0.02560	0.6		Negative			
	Visit3	1	0.21615	4.2	0.19508	Positive	1.33 (13.3)		HuS19R Titre
		2	0.15200	2.5		Negative			
		4	0.09970	8.1		Negative			
		8	0.06325	12.6		Negative			
		16	0.06965	31.2		Negative			
		32	0.03650	14.3		Negative			
		64	0.04705	26.6		Negative			
		128	0.02425	10.2		Negative			
	Visit7	1	0.42185	4.2	0.16919	Positive	3.28 (32.8)		HuS33 Titre
		2	0.23500	3.2		Positive			
		4	0.13240	3.4		Negative			
		8	0.07080	1.8		Negative			
		16	0.04280	17.5		Negative			
		32	0.02265	7.8		Negative			
		64	0.01600	2.7		Negative			
		128	0.01245	10.8		Negative			

MRD = Method requires a 10 fold dilution prior to analysis

* Calculated using decisive NF determined from pre-dose (visit 2) samples

14.2.4.1.2 Listing of pre-existing ADA-positive patients: Titer and boosting, FAS

Treatment	Subject ID	Visit	Parameter	Result
dasiglucagon	(b) (6)	Day 0	ADA Dasiglucagon Screening test	Positive
			ADA Dasiglucagon Confirmatory test	Positive
			ADA Dasiglucagon Titre	1.40
			ADA GlucaGen Confirmatory test	Negative
		Day 7	ADA Dasiglucagon Screening test	Positive
			ADA Dasiglucagon Confirmatory test	Positive
			ADA Dasiglucagon Titre	1.33
			ADA GlucaGen Confirmatory test	Negative
		Day 14	ADA Dasiglucagon Screening test	Positive
			ADA Dasiglucagon Confirmatory test	Negative
		Day 35	ADA Dasiglucagon Screening test	Positive
			ADA Dasiglucagon Confirmatory test	Negative
		Day 104	ADA Dasiglucagon Screening test	Positive
			ADA Dasiglucagon Confirmatory test	Positive
			ADA Dasiglucagon Titre	3.28
			ADA GlucaGen Confirmatory test	Negative

The increase in the dasiglucagon titer from baseline (≤ 2.3 -fold increase) was within expected assay variability and did not meet pre-defined criteria for treatment-boosted ADA response (≥ 4 -fold increase in ADA titer from baseline, see section 9.5.1.1 of integrated summary of immunogenicity), therefore was not considered significant. These 3 confirmed ADA+ samples from subject (b) (6), were further tested for cross-reactivity with glucagon but the cross-reactivity results towards endogenous glucagon was negative (Table 11b).

Table 11b: cross reactivity to endogenous glucagon (for samples confirmed ADA+ to anti-ZP4207 antibodies)

Subject	Visit	Mean absorbance with glucagon	Mean absorbance without glucagon	Ratio	Recalculated Plate specific CP*	Result	Date of Analysis	Run ID
(b) (6)	Visit2pd	0.08110	0.06775	1.20	0.14231	Negative	(b) (6)	HuS34 CON
	Visit3	0.09655	0.11225	0.860	0.14231	Negative		HuS34 CON
	Visit7	0.13915	0.15000	0.928	0.14231	Negative		HuS34 CON

Confirmatory CP = 0.767265

* Calculated using decisive NF determined from pre-dose (visit 2) samples

All three of these samples were also analysed at the same time in a titre determination run for anti-glucagon (HuS35 Titre) at the Sponsor's request. However as all samples were confirmed negative for anti-glucagon, data from the titre determination has not been reported.

[Reviewer Note: The footnote in table 11b indicates that the sponsor used a CCP of 0.76727 in analyzing the anti-glucagon antibody results, which was determined during assay validation study using 0.1% FPR, but the sponsor later clarified that a new CCP of 0.821605 (determined using 1% FPR) was used for glucagon antibody assay and re-evaluated the assay results manually to determine the anti-glucagon antibody-positive samples. However, they did not update the footnote in table 3 (ISI, Study Number (b) (4)/081) with that information (see § 8 Analysis of immunogenicity data – summary of clinical data above). The reevaluated results using the newly calculated CCP at 1% FPR did not change the final results.]

Reviewer comment: Three clinical samples (pre-dose, visit 3 and visit 7) from one subject (Subject (b) (6)) treated with 0.6 mg dasiglucagon were confirmed positive for anti-ZP4207 antibody positive. The antibody titer in samples from visit 3 to visit 7 did not significantly change in compared to the baseline titer, hence the anti-ZP4207 ADA response for subject (b) (6) was not classified as a treatment-boosted response according to the trial protocol (clinical protocol: ZP4207-16136, 21 August 2017). The confirmed ADA+ samples were negative when tested for cross-reactivity to endogenous glucagon. The results from the anti-glucagon analysis showed that no samples were confirmed treatment-induced ADA+ from clinical trial ZP4207-16136.

Overall, there were no subjects from clinical trial #16136 with a treatment-induced or treatment-boosted antibody response. Therefore, the PK, PD, and other safety parameters are not expected to be impacted due to immunogenicity in these subjects.

f. Study: ZP4207-16137 (Phase 3, (b) (4) study number (b) (4)/093)

This was a single-dose *parallel arm* trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in patients with T1DM. The patients were randomized at 2:1:1 to receive single SC dose of dasiglucagon (0.6mg), GlucaGen® (1.0mg)

or placebo. Samples were analyzed for the development of ADAs in samples at pre-dose and at 28 days after dosing.

Results: Of 168 samples, 14 samples were screened positive for anti-ZP4207 antibodies, 3 of 14 putative ADA+ samples from 2 subjects (Subjects (b) (6)) were confirmed ADA+ (Table 12a). Hence, the false-positive rate was 6.5% (11/168). The visit 3 and an unscheduled FU samples from subject (b) (6) were screened positive (Table 12b) for cross-reactivity by anti-glucagon antibody assay but tested negative in confirmatory assay (Table 12c).

Eighty-six samples were tested by anti-glucagon antibody assay, five of which were screened positive, none were tested confirmed positive (FPR=5/86, 5.8%). None of the samples were also tested positive for the presence of anti-glucagon antibodies from clinical trial ZP4207-16137.

Table 12a: Confirmatory assay results for anti-ZP4207 in serum samples from Clinical Trial ZP4207-16137 (re-numbered sponsor's Table 6, Study number (b) (4)/093)

Subject	Visit	Mean absorbance with ZP4207	Mean absorbance without ZP4207	Ratio	Plate specific CP	Result	Date of Analysis	Run ID
(b) (6)	3	0.25710	0.25205	1.02	0.24078	Negative	(b) (6)	HuS11 CON
	2	0.22965	0.24280	0.946	0.24078	Negative		HuS11 CON
	3	0.22155	0.21800	1.02	0.24078	Negative		HuS11 CON
	2	0.41295	0.40845	1.01	0.24078	Negative		HuS11 CON
	3	0.40360	0.49425	0.817	0.24078	Negative		HuS11 CON
	2	0.21260	0.20155	1.05	0.24078	Negative		HuS11 CON
	3	0.25340	0.24730	1.02	0.24078	Negative		HuS11 CON
	3	0.20465	0.21335	0.959	0.24078	Negative		HuS11 CON
	2	0.20425	0.22460	0.909	0.24078	Negative		HuS11 CON
	3	0.19400	0.21705	0.894	0.24078	Negative		HuS11 CON
	2	0.12125	0.20775	0.584	0.24078	Positive		HuS11 CON
	3	0.15420	0.24835	0.621	0.24078	Negative		HuS11 CON
	3	0.14155	0.25955	0.545	0.24078	Positive		HuS11 CON
	Unscheduled	0.15285	0.36935	0.414	0.23620	Positive		HuS18A CON

Confirmatory CP = 0.612478

One of 3 samples confirmed ADA+ for anti-ZP4207 antibodies was pre-dose (Visit2) sample from subject (b) (6), and two samples were from subject (b) (6) (visit 3 and FU). The pre-dose (visit 2) sample from this subject tested ADA-negative (Sponsor's Table 5, study number (b) (4) 093, was not copied here) suggesting that the anti-ZP4207 antibodies in subject (b) (6) may have been treatment induced. This subject was ADA+ at FU visit (28 days) with a titer of 24.4. Another FU samples tested after 14 months of dosing was also found to be positive with a titer of 27.7 but was negative in next FU visit at 17 months after dosing. This Visit 3 sample from subject (b) (6) was tested negative for cross-reactivity towards endogenous glucagon (DARRTS Reference ID: 4449760, 6/17/2019) and there were no neutralization activity detected for this sample (ISI, page 67).

Table 12b: Cross reactivity (screening) to glucagon (samples confirmed ADA+ to anti-ZP4207 antibodies), sponsor's table 3 - study number (b) (4)/093.

Subject	Visit	Mean absorbance	Recalculated Plate specific CP*	Result	Date of Analysis	Run ID
(b) (6)	3	0.34665	0.13299	Screened Positive	(b) (6)	HuS14R
	Unscheduled	0.30520	0.16680	Screened Positive		HuS19

* Calculated using NF determined from pre-dose (visit 2) samples

Table 12c: Cross reactivity (confirmed) to glucagon (samples confirmed ADA+ to anti-ZP4207 antibodies), sponsor's table 4 - study number (b) (4) 093

Subject	Visit	Mean absorbance with glucagon	Mean absorbance without glucagon	Ratio	Recalculated Plate specific CP*	Result	Date of Analysis	Run ID
(b) (6)	3	0.23790	0.24260	0.981	0.09918	Negative	(b) (6)	HuS15R CON
	Unscheduled	0.35270	0.35790	0.985	0.13614	Negative		HuS20 CON

Confirmatory CP = 0.821605

* Calculated using NF determined from pre-dose (visit 2) samples

Reviewer comment: One subject (b) (6) tested positive for the presence of treatment-induced anti-ZP4207 antibodies and remained ADA+ until about 17-month of dosing when it was tested negative finally indicating that a single dose may be able to produce a sustained level of antibody to dasiglucagon. Nevertheless, the titer of the ADAs was low, and tested negative for cross-reactivity towards endogenous glucagon and no neutralization activity (ISI, page 67). Therefore, ADA mediated safety issues may not be expected.

According to the sponsor this patient experienced mild, temporary events of nausea and vomiting approximately 2 and 2.5 hours after administration of dasiglucagon. This was a single dose trial and the induction of antibodies require at least 2-3 weeks for IgM and about 4-weeks for the development of IgG, therefore ADA is not expected to affect PK or PD and be associated with this event on the dosing day. The sponsor stated that there were no clinically significant abnormal vital sign measurements, physical examination results and ECG and laboratory results, and no adverse events were reported during the follow-up period for this patient.

g. Study: ZP4207-17084 (Phase 3, bridging, (b) (4) study number-(b) (4)/125)

This was a bridging trial and conducted in 90 patients with T1DM in a single dose (0.6mg) of two dasiglucagon batches (Batches A and B). Incidence of ADAs were tested at visit 2 (pre-dose), visit 3 (day 28) and at FU visit (day 56+5 days).

Results: A total of 265 samples were tested for the presence of anti-ZP4207 antibodies, 55 samples screened positive, but none were tested ADA+ in the confirmatory assay (FPR = 55/265, 20.8%).

Reviewer comment: The false positive rate for the assay was 20.8% (55/265), which is higher FPR than generally expected (expected ~ 2-11%) for an ADA binding assay. The reason of high FPR is unknown. The sponsor used additional human IgG and IgM detection controls in this study, determined assay precision demonstrating the reliability of the assay. The higher FPR results do not appear to be related to the technical discrepancy of the assay. Visual inspection of each of the 55 samples indicates that at least 15 samples had mean absorbance value closer to

PSCP (within 10% range of the CP), could have screened as false due to screening assay variability. Nevertheless, high FPR did not increase the risk of not identifying a true positive sample by subsequent confirmatory assay, therefore did not have negative impact on the study results. Additionally, the overall FPR for all studies was within the acceptable range for both ADA screening assays (Table 17). The results from the anti-ZP4207 antibody assay showed that no samples were confirmed ADA+ from clinical trial ZP4207-17084 with a treatment induced or treatment boosted antibody response.

The aim of this trial was to evaluate safety of a single subcutaneous dose of dasiglucagon stored at the intended dual storage condition (Batch B⁸) relative to a dasiglucagon batch stored at refrigerated conditions (Batch A) for the treatment of hypoglycemia in patients with T1DM. This trial also serves to clinically qualify the impurities after dual storage conditions proposed for the marketing. The sponsor stated that the T1DM patients in this trial was exposed to dasiglucagon drug product with an impurity profile representative of the impurity profile at the proposed end-of-shelf-life. Since no confirmed ADA-positive subjects were detected from this study (trial 17084), the representative impurity profile up to the limit reported in this drug product lot (Batch TD0059) is expected to be safe with respect to ADA formation.

[Reviewer Note: For anti-dasiglucagon antibody assay, the sponsor used the NC, the NF (0.05346) and CCP established using T1DM samples in (b) (4) /115 (Partial Validation, Ref: §4.1.1 anti-ZP4207 antibody binding (BAb) assay, page 6 of this memo) and compared the pre-dose sample data of clinical subjects from this trial and the data obtained from population used in validation study (b) (4) /119). The means of the two data sets were found to be significantly different so a study specific cut point and NF were calculated based on all the Visit 2 pre-dose samples, and all samples were reevaluated based on the calculated NF of 0.02461.

Six outliers identified; F-Test for equal variances, $P=0.072$, therefore assume equal variances. T-Test (assuming equal variances), $P < 0.0001$, therefore it was concluded that the two means were significantly different. Therefore, cut point recalculated from visit 2 pre-dose samples (excluding outliers) and found the remaining data were normally distributed as $P=0.6140$ for Kolmogorov-Smirnov test. Therefore, $CP = \text{mean} + (1.645 \times SD)$ was used.

$CP = 0.1052 + (1.645 \times 0.03070) = 0.15570$; Mean NC = 0.13109 (from HuS01R, HuS02R and HuS03R)

$NF = CP - \text{global mean of NC}$; $NF = 0.15570 - 0.13109 = 0.02461$

The anti-ZP4207 CCP = 0.612478]

h. Study: ZP4207-17086 (Phase 3, pediatric pivotal, (b) (4) study number-(b) (4) /110)

⁸ *Storage of dasiglucagon drug product both at refrigerated and at room temperature for prolonged periods. Dasiglucagon should be stored in a refrigerator, 2°C to 8°C (36°F to 46°F). During the shelf-life, the drug product can be kept at room temperature up to 25°C [77°F]) for a single period of up to 12 months. The levels of impurities assayed were generally higher in the Batch B drug product, but still within the proposed DP shelf-life limits.

This was a single dose *parallel-arm trial* subjected to assess the efficacy, safety, and pharmacokinetics of dasiglucagon relative to placebo and GlucaGen® randomized at 2:1:1 as a rescue therapy for severe hypoglycemia in children with T1DM. The subjects were dosed at 0.6mg dasiglucagon or placebo or 1.0mg glucagon subcutaneously. The serum samples were tested for anti-dasiglucagon and anti-glucagon antibodies at pre-dose and at FU level.

Results: In this study, 42 samples from 20 subjects were screened for anti-ZP4207 antibodies, 7 of 42 samples were screened positive and 3 of these samples were confirmed ADA+ for anti-ZP4207 antibodies (FPR= 4/42, 9.5%). All 3 confirmed ADA+ samples were from same subject, (b) (6) (Table 13a). The samples from visit 3 and from two unscheduled FU visit were ADA+, indicating a treatment induced response.

Table 13a: Confirmatory assay results for anti-ZP4207 in samples from Clinical Trial ZP4207-17086

Subject	Visit	Mean absorbance with ZP4207	Mean absorbance without ZP4207	Ratio	Re-Calculated Plate Specific Cut Point*	Result	Date of Analysis	Run ID
(b) (6)	3	0.19915	0.22000	0.905	0.24495	NEGATIVE	(b) (6)	HuS04 CON
	3	0.45625	0.45180	1.01	0.24495	NEGATIVE		HuS04 CON
	1	0.29735	0.43790	0.679	0.24495	NEGATIVE		HuS04 CON
	3	0.25165	0.42720	0.589	0.24495	POSITIVE		HuS04 CON
	Follow-up	0.27195	0.51610	0.527	0.23689	POSITIVE		HuS09 CON
	Follow-up 2	0.20715	0.39035	0.531	0.26785	POSITIVE		HuS14R CON
	1	0.19490	0.20430	0.954	0.24495	NEGATIVE		HuS04 CON

Confirmatory CP = 0.612478

*Calculated using decisive NF determined from Visit 1 screening (pre-dose) samples

The ADA titer was determined to be 75.7 at visit 3 (Day 28 after dose), 31.1 at the unscheduled FU visit at 12-month for this subject (b) (6) (Table 13b and Table 14.3.5.6 of ISI report). This subject was tested again in May 2020, after 18 month of last dose and found still ADA+ with a titer of 30.5 (Communication on 07/24/2020 for safety update; Supporting Document Number:16). These samples were tested negative for cross-reactivity towards endogenous glucagon.

The Visit 3 sample was also tested negative for neutralizing activity. The sponsor did not report the NAb assay results for other FU samples for this ADA+ patient. On July 22, 2020, FDA sent a request for submitting further information for this ADA+ patient from trial ZP4207-17086. The sponsor provided an update on August 31, 2020 (SDN 024) in response to our request indicating that the other FU samples, including the 18-month FU sample were tested negative for NAb activity, however this subject remains ADA+ for anti-dasiglucagon antibodies after 18-month of dosing with a titer of 30.5. The results are summarized in Table 13c.

Table 13b: Titer of Confirmed ADA+ Samples from Clinical Trial ZP4207-17086

Subject	Visit	Dilution Factor	Mean Absorbance	%CV	Re-Calculated Plate Specific Cut Point*	Result	Titre (Titre x MRD)	Date of Analysis	Run ID
(b) (6)	3	1	0.59800	1.8	0.27091	POSITIVE	7.57 (75.7)	(b) (6)	HuS05 TITRE
		2	0.59750	3.2		POSITIVE			
		4	0.42930	3.0		POSITIVE			
		8	0.25170	2.2		NEGATIVE			
		16	0.11680	2.7		NEGATIVE			
		32	0.05480	0.8		NEGATIVE			
		64	0.02830	0.5		NEGATIVE			
		128	0.01475	14.9		NEGATIVE			
	Follow-up	1	0.34215	2.7	0.25117	POSITIVE	3.11 (31.1)		HuS10 TITRE
		2	0.31115	0.7		POSITIVE			
		4	0.20355	2.4		NEGATIVE			
		8	0.10440	0.5		NEGATIVE			
		16	0.05090	3.3		NEGATIVE			
		32	0.02810	1.0		NEGATIVE			
		64	0.01725	7.0		NEGATIVE			
		128	0.01275	0.6		NEGATIVE			
	Follow-up 2	1	0.34945	1.6	0.25421	POSITIVE	3.05 (30.5)		HuS15 TITRE
		2	0.30995	2.9		POSITIVE			
		4	0.20375	16.3		NEGATIVE			
		8	0.08910	10.5		NEGATIVE			
		16	0.04100	1.4		NEGATIVE			
		32	0.02430	5.2		NEGATIVE			
		64	0.01415	4.5		NEGATIVE			
		128	0.00925	19.1		NEGATIVE			

MRD = Method requires a 10 fold dilution prior to analysis
 *Calculated using decisive NF determined from Visit 1 screening (pre-dose) samples

Table 13c: Anti-dasiglucagon antibody assessments for Subject (b) (6) in trial 17086

Trial ID/ Subject ID	Baseline	Visit day/week/months	Visit day/week/months	Visit day/week/months
17086/ (b) (6)	ADA negative	Day 28: Anti-dasiglucagon antibody positive Titer 75.7 No neutralizing activity	12 months ¹ Anti-dasiglucagon antibody positive Titer 31.1 No neutralizing activity	18 months ¹ Anti-dasiglucagon antibody positive Titer 30.5 No neutralizing activity
		Anti-glucagon antibody negative	Anti-glucagon antibody negative	Anti-glucagon antibody negative

Footnotes: ¹ months since Day 28 assessment; Abbreviations: ADA: anti-drug antibody;

Anti-glucagon antibody assay results: Twenty-three samples from 11 subjects were screened in the anti-glucagon antibody assay, 5 samples were screened positive for glucagon-specific antibodies (Table 13d) and one of these 5 samples (from Patient (b) (6)) was confirmed positive for anti-glucagon antibodies which was a pre-dose sample (FPR=4/23, 17.4%). The FPR for the anti-glucagon antibody screening assay is higher than the expected (FPR of 2-11%). Nevertheless, FPR is acceptable since true ADA+ samples could be identified using the confirmatory assay and high FPR should not have a negative impact on the confirmatory assessment of the study. Additionally, the overall FPR from all studies was found to be within the general acceptable range of the FPR (2-11%) for both ADA screening assays, which is summarized in [Table 17](#) below.

Table 13d: anti-glucagon antibodies in samples from Trial ZP4207-17086

Subject	Visit	Mean Absorbance with Glucagon	Mean Absorbance without Glucagon	Ratio	Plate Specific Cut Point	Result	Date of Analysis	Run ID
(b) (6)	3	0.13670	0.14655	0.933	0.16508	NEGATIVE	(b) (6)	HuS06 CON
	3	0.15730	0.16180	0.972	0.16508	NEGATIVE		HuS06 CON
	Follow-up	0.14105	0.14830	0.951	0.16175	NEGATIVE		HuS12 CON
	1	0.13615	0.15675	0.869	0.16508	NEGATIVE		HuS06 CON
	1	0.07120	0.18040	0.395	0.16508	POSITIVE		HuS06 CON

Confirmatory CP = 0.821605

*Subject tested positive for treatment-induced anti-ZP4207 antibodies, therefore cross-reactivity with endogenous glucagon was tested

The sponsor determined the antibody titer of this anti-glucagon antibody-positive sample. During titrating assay, none of the dilutions tested (including MRD) generated response above the plate specific cut point (PSCP; Table 13e). Hence, this result cannot be considered to be a treatment-induced or treatment-boosted anti-glucagon antibody response.

Table 13e: Titer of confirmed ADA+ samples from clinical trial ZP4207-17086

Subject	Visit	Dilution Factor	Mean Absorbance	%CV	Plate Specific Cut Point	Result	Titre	Date of Analysis	Run ID
(b) (6)	1	1	0.13480	11.9	0.14925	NEGATIVE	Titre unable to be determined	(b) (6)	HuS07 TITRE
		2	0.07610	8.0		NEGATIVE			
		4	0.03860	25.6		NEGATIVE			
		8	0.02565	19.0		NEGATIVE			
		16	0.01230	4.6		NEGATIVE			
		32	0.00705	1.0		NEGATIVE			
		64	0.01030	42.6		NEGATIVE			
		128	0.00845	66.1		NEGATIVE			

MRD = Method requires a 10-fold dilution prior to analysis

Reviewer comment: The results from the anti-ZP4207 antibody analysis showed that one subject (Subject (b) (6)) treated with 0.6 mg dasiglucagon was anti-ZP4207 antibody positive at FU visit (Visit 3) but not at pre-dose (Visit 1), indicating a treatment induced response. An unscheduled FU sample from this subject was also tested positive for anti-ZP4207 antibodies, with a lower titer than the visit 3 sample. These samples were tested negative for neutralizing activity and for cross-reactivity to endogenous glucagon.

The results from the anti-glucagon analysis showed that the pre-dose sample of Subject (b) (6) treated with 1 mg GlucaGen® was anti-glucagon positive but not at FU (Visit 3). Therefore, treatment boosted, or treatment induced response was not observed.

Overall, there was one subject from clinical trial ZP4207-17086 with a treatment induced anti ZP4207 response and no subjects had a treatment induced or treatment boosted anti-glucagon antibody response.

[Reviewer Note: The NC and cut point used were established using T1DM samples from clinical trial ZP4207-16137 (b) (4)/093) in (b) (4)/119. PSCP (floating CP) = mean assay NC + 0.05346 (NF), CCP = 0.612478 for anti-dasiglucagon antibody assay.

The sponsor used pre-dose samples from current study and analyzed to determine if there was a statistically significant difference between the population used in study (b) (4)/115 and the

patients in the clinical trial. The patient's data were also compared to the subjects from study (b) (4)/119 and the means were not found to be significantly different (data not shown) so the NF used was 0.05346. However, using this NF approximately 38% of the samples screened positive. Therefore, a new study-specific cut point and NF was re-calculated based on the pre-dose from subjects in this trial ZP4207-17086. Study specific NF = 0.13161, PSCP (floating CP) = mean assay NC + 0.13161 to screen putatively positive samples. CCP = 0.612478.

For anti-glucagon antibody assay, the sponsor determined a NF using serum samples from trial subjects (see comment under § 4.1.2 anti-Glucagon antibody binding (BAb) assay, page 7 of this memo). The sponsor analyzed all pre-dose samples from this study using an appropriate t-test and determined that there was no statistically significant difference between the population used in the validation ((b) (4)/036) and the patients in the clinical trial, therefore the NF 0.05622 was used in this evaluation study.

No outliers identified; F-Test for equal variances, P=0.006, therefore assume unequal variances. T-Test (assuming unequal variances), P value was 0.3186 therefore, it was concluded that the two means are not significantly different. Same CP as used in validation study ((b) (4) 036 used.]

- i. Study: ZP4207-17145 (Phase 3, pivotal, (b) (4) study number – (b) (4) 120)

This was a single-dose *parallel-group* trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with T1DM. The mean absorbance of the study samples from clinical study ZP4207-17145 are submitted in study number (b) (4)/120. Antibodies against dasiglucagon were measured at the dosing visit (Visit 2) prior to injection and at the follow-up visit (Visit 3).

Results: A total of 68 samples were assessed and 5 samples (shown in Table 14a) were screened positive for anti-ZP4207 antibodies but none of these samples tested positive in the confirmatory assay. Therefore, these samples are considered as false-positive (FPR=5/68, 7.4%).

Table 14a: Confirmatory assay results for anti-ZP4207 in samples from Clinical Trial ZP4207-17145

Subject	Visit	Mean absorbance with ZP4207	Mean absorbance without ZP4207	Ratio	Dilution	Plate Specific Cut Point	Result	Date of Analysis	Run ID
(b) (6)	2	0.92750	0.92220	1.01	1	0.26596	Negative	(b) (6)	HuS04 CON
	3	0.87795	0.90260	0.973	1	0.26596	Negative		HuS04 CON
	2	0.54140	0.53045	1.02	2	0.29144	Negative		HuS05 CON
	3	0.60500	0.60300	1.00	2	0.29144	Negative		HuS05 CON
	3	0.30260	0.33515	0.903	1	0.26596	Negative		HuS04 CON
	2	0.40620	0.43720	0.929	1	0.26596	Negative		HuS04 CON
	3	0.42255	0.46740	0.904	1	0.26596	Negative		HuS04 CON

Confirmatory CP = 0.612478

*For Subject (b) (6) reassay with dilution was required for both visit 2 and 3 samples as the mean absorbances in run HuS04 CON exceeded that of the HPC samples. Both sets of results have been presented.

Reviewer comment: The false positive rate for the ADA assay in samples from Clinical Trial ZP4207-17145 was 7.4% for the anti-ZP4207 assay which is within an acceptable range of 2-11%. The sponsor used human IgG and IgM detection control samples as per the Study Plan in

each batch analysis. Mean absorbance for the detection controls for the anti-ZP4207 runs are provided (not copied here). The inter-assay precision of the IgG and IgM LDC, MDC and HDC met the acceptance criteria. The results from the anti-ZP4207 analysis showed that no samples were confirmed ADA+ from clinical trial ZP4207-17145.

j. Study: ZP4207-16051 (Phase 2, pump (b) (4) study number- (b) (4) /072)

This study was a *two-part cross-over study*, where adult patients with T1DM were enrolled to assess the safety and efficacy of the bihormonal bionic pancreas (BP) using the iPhone platform, using ZP4207 and Lilly's GlucaGen, where dasiglucagon or Lilly's glucagon delivered via pump. This (b) (4) study results are provided as a supportive data for immunogenicity of dasiglucagon for this NDA.

In Part 1 of the study using the iPhone platform, a total of 10 subjects with T1DM were exposed to dasiglucagon and 12 subjects were exposed to glucagon. The assay CP was determined from pre-dose samples using mean of the sample signals + 1.645 x SD for the anti-ZP4207 and anti-glucagon antibody binding assay. The Part 2 of the trial using the iLet (a wearable medical device designed to autonomously control glucose level in patients) was not conducted due to unavailability of the iLet (Clinical Trial Report ZP4207-16051; Trial Design and Plan).

ADA assessment for serum anti-ZP4207 and anti-glucagon antibodies, was conducted on samples at pre-dose, at Visit 5 (10 days ±3 days following the last day of dosing (Visit 4) and at follow-up visit 6 (25 days ±4 days following the last day of dosing).

Results: A total of 33 clinical samples were tested for ADA, 6 of 33 samples were tested positive by the screening assay for anti-ZP4207 antibodies, but all tested negative in the confirmatory assay (FPR= 6/33, 18.2%; Table 15a).

These samples were also tested by anti-glucagon antibody assay, 6 of 33 samples were screened positive for anti-glucagon antibodies, but all scored negative in the confirmatory assay (FPR=6/33, 18.2%; Table 15b).

Table 15a: Confirmatory assay results for anti-ZP4207 in serum samples of subjects from Trial 16051

Subject	Visit	Mean absorbance with ZP4207	Mean absorbance without ZP4207	Ratio	Plate specific CP	Result	Date of Analysis	Run ID
(b) (6)	3a	0.25605	0.27545	0.930	0.27128	Negative	(b) (6)	HuS03 CON
	5	0.22925	0.24175	0.948	0.27128	Negative		HuS03 CON
	6	0.23755	0.26465	0.898	0.27128	Negative		HuS03 CON
	3a	0.14715	0.12200	1.21	0.27128	Negative		HuS03 CON
	5	0.15450	0.13575	1.14	0.27128	Negative		HuS03 CON
	6	0.14825	0.15520	0.955	0.27128	Negative		HuS03 CON

Confirmatory CP = 0.511094

Table 15b: Confirmatory assay results for anti-glucagon in serum samples of subjects from Trial 16051

Subject	Visit	Mean absorbance with glucagon	Mean absorbance without glucagon	Ratio	Plate specific CP	Result	Date of Analysis	Run ID
(b) (6)	3a	0.18530	0.20425	0.907	0.19337	Negative	(b) (6)	HuS04R CON
	5	0.19695	0.17735	1.11	0.19337	Negative		HuS04R CON
	6	0.20110	0.22580	0.891	0.19337	Negative		HuS04R CON
	3a	0.10810	0.11050	0.978	0.19337	Negative		HuS04R CON
	5	0.12915	0.13650	0.946	0.19337	Negative		HuS04R CON
	6	0.12800	0.13580	0.943	0.19337	Negative		HuS04R CON

Confirmatory CP = 0.767265

[Reviewer Note: The footnote in tables 15a and 15b indicate that the CCPs for dasiglucagon and glucagon antibody binding assays, used in analyzing the results, were determined during assay validation study using 0.1% FPR. The sponsor later confirmed (response to FDA IR comment dated 10/09/2020) that they recalculated the CCP to 0.612478 for dasiglucagon and to 0.821605 for glucagon antibody assay using 1% FPR and re-evaluated the results manually but did not update the footnotes in these tables (see §8 Analysis of immunogenicity data – summary of clinical data, above). The results did not change as a result of using the new CCP determined using 1% FPR.]

Reviewer comment: The FPR for both assays were higher (18.2%) than expected. The reason is unclear, the sample size was small, may have affected the statistical analysis of the data. Nevertheless, high FPR did not increase the risk of not identifying a true positive sample since the putative ADA+ samples are further tested by confirmatory assay, therefore did not have negative impact on the study results. Additionally, the overall FPR from all studies was within the acceptable range of FPR for both ADA screening assays (Table 17). No samples confirmed positive for anti-ZP4207 or anti-glucagon antibodies from any samples from clinical study ZP4207-16051.

[Reviewer Note: Samples were initially analyzed using the screening assay for anti-ZP4207 antibodies (Ref: §4.1.1 anti-ZP4207 antibody binding (BAb) assay, page 6 of this memo). Normalization factor (NF) for the screening assay used = 0.086381.

After screening the pre-dose samples from all subjects, the data were compared against the data from individuals screened in the validation study ((b) (4) 037) that were used to calculate the screening CP. Outliers were removed by using box-plot and the samples were determined by evaluating the distribution of the absorbance values. The mean OD values from the pre-dose samples and the samples used in the calculation of the screening cut-point were compared, excluding all outliers. The P value was less than 0.05 ($P < 0.0001$) therefore, it was concluded that the two means are significantly different. Therefore, the CP was recalculated from pre-dose samples from this study (excluding outlier), the data were normally distributed ($P = 0.7197$ for D'Agostino-Pearson test), therefore the screening CP was calculated using mean of the remaining values + 1.645 times the standard deviation.

$CP = 0.06199 + (1.645 \times 0.01700) = 0.089955$; Global mean NC = 0.1108375 (from HuS01R)

Similarly, for the anti-glucagon antibody assay, the means for the pre-dose samples data from all subjects were compared with the mean data from individuals screened in the validation study ((b) (4) 036) and found statistically different. Therefore, a new NF and CP were determined using pre-dose samples (excluding outlier), which were normally distributed (P=0.1408 for D’Agostino-Pearson test), therefore the screening CP was calculated using mean of the remaining values + 1.645 times the standard deviation.

$$CP = 0.1102 + (1.645 \times 0.03563) = 0.16881135; \text{ Global mean NC} = 0.1578625 \text{ (from HuS02R)}$$

k. Study: ZP4207-16098 (Phase 2, low range, (b) (4) study number-(b) (4)/076)

This was a multiple-dose, phase 2, (b) (4) study (b) (4). The trial was a *cross-over* (with glucagon) and randomized study, with 4 dosing visits (V2, V3, V4 and V5) and a FU visit (V6). Dasiglucagon was administered at 0.03, 0.08, 0.2 and 0.6mg and glucagon was dosed at 0.03, 0.08 and 0.2mg concentrations. Dosing visits were 2 days each and each dosing visits (V2-V5) were separated by 3-7 days. The immunogenicity results from this trial was submitted to support the immunogenicity of dasiglucagon for the rescue treatment of diabetic patients under this NDA.

The immunogenicity data of 23 subjects were reported at pre-dose, visit 5 (21 days after first dose) and visit 6 (FU, 21 ± 3 days after Visit 5). The feasibility of administering dasiglucagon was evaluated in T1DM patients via a pump under euglycemic and hypoglycemic conditions.

Results: Of 64, 7 samples were screened ADA+ for anti-ZP4207 and 2 samples were confirmed ADA+. Both samples were from one subject (b) (6) (Table 16a; FPR=5/64, 7.8%) and were from 4 weeks FU and at 3.5month FU studies. This subject tested negative after 7-month of dosing.

On the other hand, 8 samples from subjects (b) (6) were screened ADA+ for anti-glucagon antibodies and one sample (Visit 6 FU) from patient 203 was confirmed for the anti-glucagon antibody (FPR=7/64, 10.9%). The same sample from subject (b) (6) was also confirmed positive for anti-dasiglucagon antibodies (Table 16b). The titer at this visit was determined to be 35.4 for anti-ZP4207 (Table 16c) and 33.8 for anti-glucagon (Table 16d). The anti-dasiglucagon antibody at FU visit sample, 24 days after the last drug exposure was tested cross-reactive to glucagon (ISI, page 56; section 6.3.1 Efficacy and Pharmacokinetics).

Table 16a: Confirmatory assay results for anti-ZP4207 in serum samples of subjects from Trial 16098

Subject	Visit	Mean absorbance with ZP4207	Mean absorbance without ZP4207	Ratio	Plate specific CP	Result	Date of Analysis	Run ID
(b) (6)	6	0.13635	0.37535	0.363	0.31222	Positive	(b) (6)	HuS05A CON
	extra	0.08950	0.20895	0.428	0.20692	Positive		HuS12 CON
	6	0.15525	0.15250	1.02	0.24197	Negative		HuS08 CON
	2	0.15275	0.20075	0.761	0.24197	Negative		HuS08 CON
	5	0.11680	0.18265	0.639	0.24197	Negative		HuS08 CON
	6	0.14005	0.22045	0.635	0.24197	Negative		HuS08 CON
	6	0.18420	0.18160	1.01	0.24197	Negative		HuS08 CON

Confirmatory CP = 0.511094

Table 16b: Confirmatory assay results for anti-glucagon in serum samples of subjects from Trial 16098

Subject	Visit	Mean absorbance with glucagon	Mean absorbance without glucagon	Ratio	Plate specific CP	Result	Date of Analysis	Run ID
(b) (6)	2	0.12655	0.13245	0.955	0.28092	Negative	(b) (6)	HuS07 CON
	6	0.13995	0.13545	1.03	0.28092	Negative		HuS07 CON
	6	0.18120	0.19665	0.921	0.28092	Negative		HuS07 CON
	2	0.10350	0.10885	0.951	0.28092	Negative		HuS07 CON
	6	0.14840	0.13985	1.06	0.28092	Negative		HuS07 CON
	6	0.14935	0.12590	1.19	0.28092	Negative		HuS07 CON
	6	0.09960	0.16935	0.588	0.28092	Positive		HuS07 CON
	extra	0.10535	0.10200	1.03	0.16063	Negative		HuS13 CON

Confirmatory CP = 0.767265

[Reviewer Note: The footnote in tables 16a and 16b indicate that the CCPs for dasiglucagon and glucagon used in analyzing the results, were determined during assay validation study using 0.1% FPR. The sponsor later (in response to FDA IR dated 10/09/2020) confirmed that they used the revised CCP of 0.612478 for dasiglucagon and of 0.821605 for glucagon determined using 1% FPR and re-evaluated the results manually but did not update the footnotes in tables 16a and 16b (see § 8 Analysis of immunogenicity data – summary of clinical data and [appendix3](#)). The final results did not change as a result of using CCP determined at 1% FPR.]

Table 16c: Titer of confirmed positive study samples from Clinical Trial #16098 (anti-ZP4207)

Subject	Visit	Dilution factor	Mean absorbance	%CV	Recalculated Plate specific CP*	Result	Titre (Titre x MRD)	Date of Analysis	Run ID
(b) (6)	6	1	0.36770	4.8	0.16490	Positive	3.54 (35.4)	(b) (6)	HuS06 Titre
		2	0.27010	2.5		Positive			
		4	0.13325	1.5		Negative			
		8	0.09980	11.8		Negative			
		16	0.05010	8.8		Negative			
		32	0.03055	2.1		Negative			
		64	0.02520	20.2		Negative			
		128	0.01645	2.1		Negative			
	extra	1	0.25580	6.8	0.13407	Positive	3.88 (38.8)		HuS14 Titre
		2	0.21650	4.2		Positive			
		4	0.12860	4.5		Negative			
		8	0.07930	15.9		Negative			
		16	0.04790	8.3		Negative			
		32	0.02405	7.4		Negative			
64	0.01585	13.8	Negative						
128	0.01500	27.3	Negative						

Table 16d: Titer of confirmed positive study samples from Clinical Trial #16098 (anti-glucagon)

Subject	Visit	Dilution factor	Mean absorbance	%CV	Recalculated Plate specific CP*	Result	Titre (Titre x MRD)	Date of Analysis	Run ID
(b) (6)	6	1	0.12900	19.5	0.06592	Positive	3.38 (33.8)	(b) (6)	HuS09 Titre
		2	0.09110	5.0		Positive			
		4	0.05465	13.1		Negative			
		8	0.04070	10.8		Negative			
		16	0.02170	17.6		Negative			
		32	0.01420	12.9		Negative			
		64	0.01235	6.3		Negative			
		128	0.00985	15.1		Negative			

MRD = Method requires a 10 fold dilution prior to analysis

* Used to identify samples for confirmatory analysis and titre determination only. Calculated using NF determined from pre-dose samples.

Sample analysis for neutralizing antibody (NAb): The ADA+ sample (V6 FU) from subject (b) (6) was tested positive for neutralizing activity by both the anti-dasiglucagon and anti-glucagon neutralizing antibody assay (Tables 16e and 16g). The sponsor stated that there was no indication that these antibodies had an effect on PK or PD.

The NAb+ sample was diluted to determine antibody titer and assessed at 1:10, 1:20, 1:40 and 1:80 for both assays, all of the titers tested negative for NAb (Table 16f) suggesting the antibody concentration was relatively low. The Glucagon NAb assay failed to meet acceptance criteria on '21Jul17' sample due to the LPC being greater than the MPC (Table 16g). This assay was not repeated as there was no sufficient sample volume to repeat the analysis.

Table 16e : anti-ZP4207 Neutralizing antibody results

Assay Date	Name	Mean	Std Dev.	%CV	Cutpoint (1.29) Result
14Jul17	Blank	2.9399	0.01421	0.5	NA
	HPC	2.2815	0.2262	9.9	
	MPC	1.3971	0.13705	9.8	
	LPC	1.2664	0.12939	10.2	
	NC	1.0000	0.11518	11.5	
	Day 1 Predose ADA Back Up 203 Visit 2 1:10	1.0700	0.11547	10.8	Negative for Nab
	Follow Up ADA Back Up 203 Visit 6 1:10	1.6289	0.02007	1.2	Positive for NAb

Table 16f : anti-ZP4207 Neutralizing antibody titer results

Assay Date	Name	Mean	Std Dev.	%CV	Cutpoint (1.29) Result
21Jul17	Blank	2.2028	0.07201	3.3	NA
	HPC	1.8369	0.03054	1.7	
	MPC	1.4839	0.17997	12.1	
	LPC	1.1886	0.07003	5.9	
	NC	1.0000	0.08725	8.7	
	Follow Up ADA Back Up 203 Visit 6 1:20	1.1707	0.05013	4.3	Negative for NAb
	Follow Up ADA Back Up 203 Visit 6 1:40	1.0576	0.04482	4.2	Negative for NAb
	Follow Up ADA Back Up 203 Visit 6 1:80	1.0205	0.12645	12.4	Negative for NAb

Table 16g: anti-glucagon Neutralizing antibody results

Assay Date	Name	Mean	Std Dev.	%CV	Cutpoint (1.25) Result
14Jul17	Blank	3.8064	0.00245	0.1	NA
	HPC	2.9585	0.1248	4.2	
	MPC	1.6257	0.087	5.4	
	LPC	1.2101	0.12489	10.3	
	NC	1.0000	0.05374	5.4	
	Day 1 Predose ADA Back Up 203 Visit 2 1:10	1.1427	0.01817	1.6	Negative for Nab
	Follow Up ADA Back Up 203 Visit 6 1:10	1.3504	0.12105	9.0	Positive for NAb

Reviewer comment: The sponsor used additional human Immunoglobulin Detection Controls (3 dilution of each IgG and IgM) as they used anti-mouse secondary reagent in the antibody binding assays which is less-sensitive to human antibodies. The data were provided, but not

copied here (ZP4207-16098 Study Number (b) (4) 076; Table 6). The methods for the determination of anti-ZP4207 and anti-glucagon antibodies were applied successfully in analyzing samples from clinical study #16098 with a FPR of 7.8% (5/64) and 10.9% (7/64) for anti-ZP4207 and anti-glucagon assay, respectively.

Of 23, one subject (subject (b) (6)) had treatment-induced anti-ZP4207 and anti-glucagon antibodies. This subject (b) (6) (age 56, female) was administered with 8 single doses of ZP4207 and 3 single doses of glucagon over 15-21 days. ADA tested negative during treatment days but was ADA+ at FU (21 days after last and 45 days after first dose) for anti-ZP4207 (titer 35.4) and anti-Glucagon antibodies (33.8). The anti-ZP4207 antibodies in this sample was also tested positive for cross-reactivity with glucagon (§6.3.1 Efficacy and Pharmacokinetics, ISI). Due to cross-over nature of the study and high degree of amino acid sequence homology between dasiglucagon and glucagon, the ADA-positivity result could not be specifically assigned to either dasiglucagon or glucagon treatment. However, the FU sample at 3.5 months after last dose was negative for anti-glucagon but remained positive for anti-ZP4207 antibodies (titer 38.8) which could indicate for the presence of a dasiglucagon-specific epitope. Nevertheless, this subject was tested ADA-negative at the last test visit after 7-month of last dose.

The sponsor stated that during the period from the 1st till 5th dosing day the patient experienced 8 events of hypoglycemia. However, the immunogenicity samples collected during the treatment period, were tested negative for the presences of antibodies, therefore, the events of hypoglycemia during the treatment period could not be associated with the development of ADAs.

This sample was tested positive for both glucagon and dasiglucagon neutralizing activity in FU sample, 24 days after last dose, which later at 3.5 months after dosing tested negative for neutralizing activity against either dasiglucagon or glucagon. The impact of the neutralizing activity of these antibodies was unknown, however, a potential effect on the PK/PD responses at later time cannot be ruled out.

8.2 Clinical Immunogenicity results - summary

None of the subjects developed antibodies to dasiglucagon from pivotal phase 3 trial 16136 for immunogenicity. However, from all 11 clinical studies, 0.8% (4/498) patients who were exposed to dasiglucagon developed antibodies to dasiglucagon, and 0.5% (1/212) patients who received glucagon developed anti-glucagon antibodies.

8.3 False positive rates in clinical sample analysis

The cut-points for both antibody screening assays were established during the assay validation studies using 40 individual sera from healthy subjects at 95% confidence level. During the clinical sample analysis, they investigated the suitability of the validation cut-point using pre-dose serum samples from study subjects of each of 11 clinical trials reported in this application. The new plate-specific cut-point was determined from each study at 95% confidence level, as

per Agency recommendation in the determination of screening assay cut-point⁹, therefore, the ADA screening assays are expected to have a false-positive rate of about 5%. Since the sponsor determined the new plate-specific cut-point using pre-dose serum samples from trial subjects, and analyzed clinical samples, false-positive rates are calculated for each of 11 clinical studies and presented in the table 17 below.

Table 17: False positive rates in antibody screening assays in clinical studies¹⁰

Trial No	(b) (4) study number	Sponsor reference number	False positive /Total number of samples		False Positive Rate (%)	
			Anti-ZP4207 assay	Anti-Glucagon assay	Anti-ZP4207 assay	Anti-Glucagon assay
14013 Part-I (HV)	YCS-040	14-013	51/160	11/96	31.9	11.5
14013 Part-II			5/60	15/60	8.3	25
15007 (HV)	(b) (4) 048	15-007	0/48	n/a	0	n/a
15126	065	16-033	13/166	4/154	7.8	2.6
16051	072	16-078	6/33	6/33	18.2	18.2
16098	076	16-098	5/64	7/64	7.8	10.9
16136	081	16-136	67/330	9/315	20.3	2.9
16137	093	16-137	11/168	5/86	6.5	5.8
17084	125	17-084	55/265	n/a	20.8	n/a
17086	110	17-086	4/42	4/23	9.5	17.4
17144 (HV)	119	17-144	11/84	n/a	13.1	n/a
17145	120	17-145	5/68	n/a	7.4	n/a
OVERALL			233/1487	58/830	12.6	11.8

Reviewer comment: A total of 1487 clinical samples were tested for anti-dasiglucagon antibodies in dasiglucagon treated subjects, while 830 samples were analyzed for anti-glucagon antibodies in subjects treated with glucagon. Four of 12 (11+1) studies (considered 12, since trial 14103 has two parts and the sponsor reported two FPR rates for each ADA assay) resulted to higher false-positive rates (FPR) than expected in anti-ZP4207 antibody binding assay than expected (Table 17, bold numbers). The FPR generally varies between 2-11%¹¹. It is recommended that if the in-study baseline samples yielded an FPR outside these limits, a statistical comparison of the means and variances between the validation data and the in-study pre-dose baseline data should be made to further understand the differences, and a new in-study cut-point should be established. In this report, the sponsor used a new plate-specific CP determined using pre-dose study samples.

Higher FPR indicates relatively higher prevalence of ADA+ responses in the screening assay, however, the detection of true ADA+ samples will not change in the confirmatory assay due to an increase in the FPR. This will only result in a higher number of samples to be tested in the confirmatory assay. Nevertheless, the overall false-positive rates from all 11 studies are 12.6% and 11.8% for anti-ZP4207 and anti-glucagon antibody assay, which are acceptable.

9. Summary of treatment-induced ADA development:

⁹ FDA Guidance for Industry – ‘Immunogenicity Testing of Therapeutic Protein Products —Developing and Validating Assays for Anti-Drug Antibody Detection, 2019’.

¹⁰ This table was created by the reviewer from data presented in various locations in the ISI including Table 4-8 in ISI.

¹¹ Goodman, Cowen, Devanarayan et al. (2018): Feedback from the European Bioanalysis Forum: focus workshop on current analysis of immunogenicity: best practices and regulatory hurdles. Conference report, Bioanalysis Vol 10, No 4, 197-204.

Four of 498 (0.8%) subjects who received dasiglucagon during the clinical development program developed anti-dasiglucagon antibodies, whereas one of 212 subjects who received glucagon RLD, developed antibodies to glucagon. An overview of the anti-drug antibody analysis results per treatment-induced ADA positive patient is presented in Table 18 (re-created from original Table 6-4 from Integrated Summary of Immunogenicity, page 54).

According to the clinical phase 3 trial protocols, all ADA+ patients were to be followed until their antibodies returned to baseline. The sponsor followed up the status of ADAs in all ADA+ subjects and the results are summarized in Table 18. In this table, the development of the antibodies to dasiglucagon and glucagon are presented at various time-points tested in ADA+ patients from all trials administered with dasiglucagon and GlucaGen including the titer of the antibodies. The ADAs were further characterized by cross-reactive assay and neutralizing antibody assays. (This table is re-created from Table 6-4 submitted to the 'Integrated Summary of Immunogenicity (ISI), §5.3.5.3' and using additional information submitted to the application).

Table 18: Development of antibodies to dasiglucagon and glucagon

Trial	Subject ID ¹²	Baseline	Visit	Visit	Visit
15126 (Dose Finding)	(b) (6)	ADA negative	Day 7: Anti-ZP4207 antibody positive Titer ~22	Day 28 (18 days after last dose): Anti- ZP4207 antibody positive Titer ~22	ADA-negative
Anti-glucagon antibody negative			Anti-glucagon antibody negative		
16098 (cross over study (b) (4) (b) (4))		ADA negative	Day 24 (Visit 6): Anti- ZP4207 antibody positive Titer 35.4 Neutralizing activity	3.5 Months FU: Anti- ZP4207 antibody positive Titer 38.8	7 Months: ADA negative
			Anti-glucagon antibody positive Titer 33.8 Neutralizing activity	Anti-glucagon antibody Negative	
16137 (single dose study)	ADA negative	Day 28: Anti- ZP4207 antibody positive Titer 24.4 No neutralizing activity	14 Months: Anti- ZP4207 antibody positive Titer 27.7 No neutralizing activity	17 Months: ADA negative	
		Anti-glucagon antibody negative	Anti-glucagon antibody negative		
17086 (single dose study)	ADA negative	Day 28: Anti- ZP4207 antibody positive Titer 75.7 No neutralizing activity	12 Months: Anti- ZP4207 antibody positive Titer 31.1 No Neutralizing activity	18 Months ¹³ : Anti- ZP4207 antibody positive Titer 30.5 No Neutralizing activity	
		Anti-glucagon antibody negative	Anti-glucagon antibody negative	Anti-glucagon Ab negative	

*Subject (b) (6) was dosed SC in the abdominal region with 0.3 mg ZP4207 on (b) (6) and 0.5 mg glucagon on (b) (6) (b) (6). FU visit was on (b) (6) 18 days after the last dosing. Sample was taken before each dosing and at FU visit. This subject was ADA-negative initially but re calculating CCP using 1% FPR, it turned out to be ADA+ on the glucagon dosing day (5 days after dosing with dasiglucagon, may be IgM type). There was no cross-reaction to glucagon.

*Subject (b) (6) (patient number (b) (6)) was administered with 8 single doses of ZP4207 and 3 single doses of glucagon, both at doses between 0.03 and 0.6 mg, distributed over 8 treatment days over 15-21 days. The ADA was tested at first and seventh treatment days and at FU. The results for the treatment days were negative, but at FU visit, 45 days after first dosing and 24 days after last dosing, was found to be ADA+ for anti-zp4207 and anti-glucagon antibodies. A FU sample at 3.5 months (109

¹² In the Integrated Summary of Safety (Module 5.3.5.3), the sponsor stated that the screening number was used as the subject ID (footnote, page 55 of ISI).

¹³ 18 months since Day 28 assessment (Responses to FDA IR dated 22 JUL 2020 to ZP4207-17086 ADA and nAB).

days) after last dosing was negative for anti-glucagon but positive for anti-zp4207 antibodies with a titer of 38.8, similar to the previous titer. This sample was tested negative at another FU visit sample at 7 months (214 days) after last dose. In the period from the 1st dosing day prior to dosing until the 5th dosing day the patient experienced 8 events of hypoglycemia. Due to the cross-over design of this trial, the induction of ADA cannot be associated with a specific treatment. The test for neutralizing activity was found positive for both glucagon and dasiglucagon neutralizing activity.

*Subject (b) (6) in ZP4207-16137 trial, was administered one single dose of 0.6 mg dasiglucagon SC in the abdominal region on (b) (6). This subject was ADA+ at FU visit (28 days) re calculating CCP using 1% FPR as FDA suggested with a titer of 24.4. This subject was still ADA+ after 14 months after dosing with a titer of 27.7 but was negative in another FU visit at 17 months after dosing. The sponsor stated that there was no cross-reaction to glucagon or neutralizing activity.

*Subject ID (b) (6) (11 years), was administered one single dose of 0.6 mg dasiglucagon SC in the thigh, ADA samples were tested at pre-dose and at 32 days after dosing. The FU visit was ADA+ for anti- ZP4207 antibody with a titer of 75.7. An unscheduled ADA sample collected at 12 months after dosing, was also ADA+ with a titer of 31.1. An additional FU visit at 18 months was scheduled for this subject. Although the 18M sample was confirmed ADA+ for dasiglucagon, no cross-reactivity or neutralizing activity observed in this sample.

Reviewer comment: Four of 498 subjects (0.8%) developed antibodies to dasiglucagon and 1 of 212 subjects (0.5%) developed antibodies to glucagon from 11 clinical studies. The sponsor determined the antibody titer in these anti-dasiglucagon antibody positive subjects at MRD 1:10 and reported, which appears relatively low and reverts to ADA negative at later visit test in three of four cases. It is unknown to this reviewer whether additional follow up testing for Subject (b) (6) (Study 17086) is scheduled.

No adverse events that could be related to ADA were reported in any of the ADA+ patients. The ADA positive patients in trials 16098 and 16137 were ADA negative after 7 and 17 months of dosing, respectively. The ADA+ patient (b) (6) in trial 15126 who was identified after changing of CCP (to 1% FPR), was followed up until they became ADA negative. The time when this subject became ADA-negative was not reported. The follow-up result for the patient in trial 17086 is known to remain ADA+ in last visit test after 18-month of the dose.

Formation of high-titer antibodies is known to potentially influence the efficacy of drugs, either indirectly by affecting the PK (clearing or sustaining antibodies) or directly by neutralizing the drugs ability to induce a response. The consequences of clearing and/or inhibiting glucagon activity by this process, via cross-reactivity with anti-dasiglucagon ADA could in theory result in a greater risk of experiencing hypoglycemia. Therefore, the titer assay provides an understanding of the concentration of antibodies present. The greater the concentration of the specific antibody in the serum sample, the higher the titer. The titer is determined by serially diluting the serum samples containing antibodies and evaluating the sample where a positive assay response is still detectable. In the ADA+ samples from these studies, the titer is relatively low and determined at dilution equivalent to MRD; therefore, may not be expected in general to have serious adverse effect due to antibodies in the short term. However, the potential formation of sustained antibodies could significantly affect the effect of dasiglucagon, which may also have adverse reactions. For a single-dose studies at least two ADA+ subject (from studies 16137 and 17086) experienced the presence of antibodies for a period of longer than a year. The antibody titer for these subjects was low, and the impact of having sustained antibody level in these subjects are not known. Nevertheless, it appears that some subjects exposed to dasiglucagon had the risk of potential occurrence of a sustained anti-drug antibody response.

10. Treatment-boosted ADA development:

A total of five patients had pre-existing antibodies, tested by anti-dasiglucagon antibody assay. The sponsor assessed these five patients to determine if their anti-drug antibody responses were boosted after treatment with dasiglucagon. The following review table 19 summarizes the assessment results and is also sponsor Table 6-5 in the ISI.

Table19: Titer development over time in baseline ADA positive subjects (Sponsor Table 6-5 from ISI)

Trial	Subject ID	Visit	Titer (including MRD=10)	Titer increase
14013 Part 1 (HV)	(b) (6)	Predose	11.4	-
14013 Part 2 (T1DM)		Predose	16.2	-
		Visit 3	22.0	1.4x
		Visit 4	15.2	0.9x
16136		Predose	14.0	-
		Visit 3	13.3	1.0x
		Visit 7	32.8	2.3x
16137		Predose	ND*	
17144		Predose	32.1	-
		Follow up	34.9	1.1x

*Titer not tested as the visit 3 sample was negative

Reviewer comment: In order to be categorized as having a treatment-boosted antibody response, a 4-fold increase in titer was needed according to the protocol. The titer increase in these subjects did not reach to 4-fold increase compared to the baseline titer. Therefore, none of the patients were classified as a treatment-boosted antibody response. These titers appear very similar in magnitude to those reported in Table 18 as treatment-induced; however, subjects reported in the previous review section above (§9. Summary of treatment-induced ADA development) as positive for treatment-induced ADA had negative baseline (pre-dose) samples.

11. Clinical consequences of ADA formation:

The evaluation of the impact of ADA formation with PK and PD was planned to be studied in the Phase 3 immunogenicity trial 16136, where each patient was dosed with 3 weekly single doses of either dasiglucagon or GlucaGen or placebo. The protocol included an additional dosing visit for ADA+ patients for PK/PD assessments, but no patients developed ADA in this trial, hence, a correlation between the ADA formation and effect on PK / PD was not carried out.

Nevertheless, the immunogenicity was evaluated in clinical samples from the other 10 studies. A total of 4 subjects from 11 clinical studies were tested ADA+ and the ADA status of these ADA+ subjects was followed up.

One ADA+ patient from the supportive multi-dose phase 2 trial 16098 had low-titer, cross-reacting anti-dasiglucagon antibodies at the first FU visit 24 days after the last dose. The

antibodies were positive for both dasiglucagon and glucagon in vitro neutralizing activity. No indication that the antibodies had an effect on PK or PD was reported.

The other 3 ADA+ patients were from single-dose trials. PK/PD data for these 3 ADA+ patients were not available. The sponsor stated that the delayed time-course of ADA formation in these subjects prevented an evaluation of the effects of ADA on PK/PD. No immunogenicity-related AEs were reported for any of these 4 patients who tested positive for anti-dasiglucagon antibodies.

12. Conclusion:

The immunogenicity results obtained from the clinical development program where a total of 498 subjects were administered with dasiglucagon as either single dose or multiple doses demonstrated antibody development to dasiglucagon in 4 of 498 (0.8%) subjects. Comparatively, 212 subjects were administered with GlucaGen and one subject (0.5%) developed antibodies to GlucaGen. The results from these studies suggest that the risk of the development of antibodies to dasiglucagon is low in the rescue treatment of severe hypoglycemia in adults and pediatric patients with diabetes mellitus. As the number of ADA positive patients are low, the statistical analysis in determining the impact of ADA formation on efficacy and safety could not be determined appropriately by the sponsor.

13. Prescribing information (PI) labeling - Immunogenicity

The sponsor provided draft labeling for inclusion in the PI. Section 6.2 describes the immunogenicity results from trials supporting this NDA. The sponsor-provided text, below in brackets is copied from the draft PI included in the package.

[As with all therapeutic (b) (4), there is potential for immunogenicity. The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to PROPRIETARY NAME with the incidence of antibodies to other products may be misleading.

(b) (4) [see *Clinical Studies (14)*].]

Reviewer comment: The sponsor text did not provide for reporting of glucagon cross-reactivity, nor the observation of extended ADA-positivity in follow-up testing. This information on cross-reactivity is generally reported, and the information on duration of ADA response is

recommended in an upcoming draft Immunogenicity Labeling Guidance for Industry. Therefore, the following Immunogenicity labeling will be recommended to the sponsor for inclusion in their PI labeling.

“Consistent with the potentially immunogenic properties of protein and peptide pharmaceuticals, patients treated with PROPRIETARY NAME may develop anti-dasiglucagon antibodies. The detection of antibody is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody (including neutralizing antibody) positivity in an assay may be influenced by several factors including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparisons of the incidence of antibodies to PROPRIETARY NAME with the incidence of antibodies to other products may be misleading.

In clinical trials, 4/498 (<1%) of PROPRIETARY NAME-treated patients developed treatment-emergent anti-drug antibody (ADA). Two patients receiving a single PROPRIETARY NAME dose had detectable antibodies to dasiglucagon for at least 17 months after dosing. One ADA-positive patient receiving multiple doses of PROPRIETARY NAME had detectable antibodies for up to 7 months after the last dose. This patient tested positive for neutralizing activity and showed cross-reactivity against native glucagon. Although no safety or efficacy concerns were noted for these ADA-positive subjects, due to the low incidence of ADA observed it is unknown at this time whether anti-dasiglucagon antibody may affect pharmacokinetics, pharmacodynamics, safety, and/or effectiveness of the drug.”

APPENDIX 1:

Outstanding comments from previous OBP reviewer:

The immunogenicity assay validation reports were initially submitted to IND (b) (4) which was found inadequate and an information request was sent on 10/22/2018 (DARRTS Reference ID: 4338265). The response to the following comments remained formally unaddressed (DARRTS Ref: 4121026, dated 07/06/2017 and 4332386, dated 10/28/2018):

- 1) Requested for additional data to ensure that mouse secondary IgG reagent is able to sensitively detect all drug-induced human IgG subtypes and IgM, since Zealand showed that the mouse IgG has 5 to 9-fold lower affinity to human antibodies.
- 2) Requested to recalculate CCP using 1% false positive rate (FPR) per the immunogenicity guidance since Zealand used 0.1% FPR to determine CCP for both BAb assay.
- 3) Requested for providing a binding curve for the PC since LPC and HPC concentrations were closer and under 100ng/mL for both PC used in anti-ZP4207 BAb assay validation.

- 4) Requested to establish the levels of matrix interference and to develop a plan to minimize the effect, since evidence of matrix interference was observed in serum sample HU/S/X/13/343g in anti-ZP4207 BAb assay validation study.

Reviewer comments (Comment 1-4): The sponsor did not provide responses to the IR submitted under IND development on 10/22/2018 (DARRTS Reference ID: 4338265). In response to an email reminder from the Agency in October 2019, Zealand acknowledged that the responses were not formally sent to the Agency (E-mail to IND project manager Elizabeth Godwin). The responses to most comments were found in the updated final reports that the sponsor submitted with the NDA application as requested by the agency (DARRTS Reference ID: 4533848, 12/13/2019). However, during the course of reviewing the ADA validation studies submitted to this NDA, the OBP reviewer recognized some information requested by previous reviewer which remained unaddressed by the sponsor. A request was sent to address these issues along with the NDA application (DARRTS entry ID: 4533848, 12/13/2019). The sponsor satisfactorily addressed those issues ([Appendix 1 and 2](#)) in its response.

The sponsor used anti-mouse antibody reagent to detect drug-induced anti-human antibodies in the validation of antibody binding assays. The Agency disagreed and recommended using a more suitable anti-human antibody reagent instead of anti-mouse IgG as a secondary reagent for the binding assay, due to species-specific affinity differences between antibodies. Alternatively, Zealand must demonstrate that the anti-mouse reagent is able to sensitively detect each human IgG subtype and human IgM (DARRTS Ref: 4121026, dated 07/06/2017). FDA also recommended the sponsor to include additional set of sensitivity controls (human IgG and human IgM) in each plate in all ADA assays, with at least three dilution of each Ig types (DARRTS Reference ID: 4366058, 12/19/2018). Zealand performed human IgG subclass sensitivity characterization and evaluated the detection of these quality control samples for the dasiglucagon and glucagon ADA assays (Study numbers #17-126 and #18-056) using human IgG controls at 60, 100 and 5000 ng/mL and IgM controls at 300, 500 and 5000 ng/mL to demonstrate the suitability of using these detection controls in the ADA assays (studies 18-076 and 18-077; DARRTS Ref: 4221652, 2/14/2018 and 4366058; 12/19/2018). OBP assessment of this study validation was not marked completed since the agency was expecting responses to additional IR request submitted by the previous OBP reviewer (DARRTS Ref: 4338265, 10/22/2018). In response to an email reminder, Zealand stated that they never submitted a formal response to that IR (Zealand e-mail correspondence dated 12/17/2019), but the reports submitted in studies #17-126 and studies 18-076 and 18-077 (revised in March 2019), addressed most issues raised by FDA in the outstanding IR letter on 12/22/2018. The sponsor showed that mouse secondary IgG reagent is able to sensitively detect all drug-induced human IgG subtypes and IgM (DARRTS Ref: Reference ID: 4366058, 12/19/2018). Therefore, the cut-points determined using serum samples from study subjects may be used for clinical sample analysis.

Zealand re-calculated the CCP for both antibody binding assays at the rate of 1% FPR instead of 0.1% as suggested by the agency, to reduce the false negative results (amendment final study reports for #14-173 and #14-174; dated March 2019). The CCP was determined in the validation studies for anti-dasiglucagon and anti-glucagon ADA assays, but was later revised based on pre-

dose serum samples obtained from study subjects with T1DM. The revised CCP were 0.612478 for the anti-ZP4207 screening assay and 0.821605 for the anti-glucagon screening assay.

Zealand was requested to submit a binding curve for the positive control since LPC and HPC concentrations were too close and under 100ng/mL for both PC used in anti-ZP4207 BAb assay validation. They submitted a binding curve that displays the dynamic range of the positive controls (Figure 4-3 in the 'Integrated Summary of Immunogenicity §5.3.5.3, page 27, not copied here). They composed the binding curve from the results obtained in studies on the 'Determination of LPC Concentration (Table 4, validation study 14-173) and Prozone Effect study (Table 16, validation study 14-173). The HPC and LPC controls are both in relatively low end of the binding curve, however, there was no prozone effect observed in the validation study, therefore, the levels of the selected controls are adequate. The LPC ensures the reliability of the assay during routine assay performances and ensures that the assay sensitivity remains acceptable across assay runs. LPC is usually determined at 1% FPR of the assay CP to demonstrate that the LPC consistently remains positive with a 1% FPR in both screening and confirmatory tiers. Zealand calculated LPC based on the following formula $LPC = CP + 2.33 * SD$ (Study #14-173) at 1% FPR and submitted data for the determination of LPC that justifies the concentration of LPC used in this assay.

Traditionally, the concentration of HPC is chosen from the linear range of the dilution curve, that is below the upper plateau of the curve or at the higher end of the study sample range. Shankar et al (2008) argued that the use of HPC is of minimal use in ADA immunoassays (although useful in Hook effect study); therefore, HPC can be optional (G. Shankar et al. / Journal of Pharmaceutical and Biomedical Analysis 48 (2008) 1267–1281). Zealand selected the concentration of the HPC at 58.2 ng/mL based on an optical density value that consistently produced a signal of >0.5 (ideally 1.0), which is relatively at the lower end of the curve. This justification is not understandable; nevertheless, it is acceptable in the absence of any prozone effect of this PC (Table 16, validation study 14-173) and HPC has minimal effect on assay performances.

The outstanding issue regarding the matrix interference was reviewed by Mohan Manangeeswaran of OBP and communicated to the sponsor for more information as Comment 5 in a letter on 10/28/2018, based on the evidence of matrix interference observed in serum samples in anti-ZP4207 BAb and anti-glucagon assay validation studies. Revisiting the matrix study reports, we found that one of 10 serum sample lots (sample #Hu/S/X/14/246d, underlined in table 13- matrix effect below) spiked with positive control antibodies, failed to produce acceptable %recovery results in anti-dasiglucagon antibody assay. In my evaluation, the validity of this matrix interference study is acceptable since 90% of the LPC and all HPC samples produced signal above the plate-specific cut-point and 90% of LPC and HPV samples produced acceptable %recovery.

However, in the study demonstrating the matrix effect for anti-glucagon antibody binding assay, the immunogenicity team noted that 6 of 10 serum sample lot spiked with LPC and 7 of 10 sample lots spiked with HPC had higher %recovery (136% and 159% relative to the plate LPC

(22.3 ng/mL) and HPC (117 ng/mL), respectively) in the matrix interference validation study. Importantly, it was also shown that all of these samples still had signals higher than plate-specific cut-point (table 16 - matrix effect, below).

Table 13
Matrix Effects
Observed Absorbance of Anti-ZP4207 Spiked in Individual Human Serum Lots (HuSV12)

Batch ID HuSV12		Human Serum Lot Numbers										Mean (%CV)
		Hu/S/X/ 14/246b	Hu/S/X/ 14/246c	Hu/S/X/ 14/246i	Hu/S/X/ 14/246j	Hu/S/X/ 13/343g	Hu/S/X/ 14/246d	Hu/S/X/ 13/463n	Hu/S/X/ 13/434c	Hu/S/X/ 13/434n	Hu/S/X/ 13/434o	
Assay response* (mean absorbance)												
Blank	Mean absorbance*	0.09745	0.16550	0.12230	0.20705	0.11150	0.10490	0.14415	0.11360	0.19230	0.19895	0.146 (28.8)
LPC 21.8 (ng/mL)	Mean absorbance*	0.41020	0.50740	0.38280	0.48820	0.25695	0.41730	0.43940	0.45895	0.57655	0.54320	0.448 (20.2)
	% Recovery ¹	85.1	105.3	79.4	101.3	53.3	86.6	91.2	95.2	119.6	112.7	93.0 (20.2)
HPC 58.2 (ng/mL)	Mean absorbance*	0.93650	1.12895	0.79905	0.82440	0.44675	0.94265	1.16930	0.99565	1.19570	1.01685	0.946 (23.4)
	% Recovery ²	90.0	108.6	76.8	79.3	43.0	90.6	112.4	95.7	115.0	97.8	90.9 (23.3)
Plate Specific CP = 0.35789												
Mean Plate LPC absorbance (in negative control serum) = 0.482												
Mean Plate HPC absorbance (in negative control serum) = 1.04												
*Mean absorbance is mean of duplicates												
¹ % Recovery = 100*Mean absorbance /Mean Plate LPC absorbance												
² % Recovery = 100*Mean absorbance /Mean Plate HPC absorbance												
Mean and %CV data rounded to 3 significant figures												

It is unclear if the high %recovery was due to a matrix effect in some of the individual sera or was some unknown error during testing. It is worth noting here that the sponsor used the same positive control monoclonal antibody for the validation of anti-dasiglucagon antibody assay, and no matrix interference was seen in that assay. This question remained unaddressed by the sponsor until OBP requested the sponsor to address this issue in an information request letter on June 19, 2020 (DARTS Reference ID: 4627990). The sponsor adequately addressed this issue, which is reviewed under [Appendix 2](#).

Table 16
Matrix Effects
Observed Absorbance of Anti-Glucagon Spiked in Individual Human Serum Lots (HuSV11RR)

Batch ID HuSV11RR		Human Serum Lot Numbers										Mean (%CV)
		Hu/S/X/ 13/343g	Hu/S/X/ 14/246j	Hu/S/X/ 13/343h	Hu/S/X/ 13/343f	Hu/S/X/ 14/246g	Hu/S/X/ 14/246i	Hu/S/X/ 14/246d	Hu/S/X/ 13/463m	Hu/S/X/ 13/463e	Hu/S/X/ 14/246f	
Assay response* (mean absorbance)												
Blank	Mean absorbance*	0.12435 [†]	0.11000	0.08615	0.08415	0.09710	0.09270	0.07815	0.11970	0.18575	0.07080	0.105 (31.8)
LPC 22.3 (ng/mL)	Mean absorbance*	0.20200	0.23295	0.21015	0.27980	0.33010	0.20530	0.32840	0.31520	0.33470	0.28770	0.273 (20.2)
	% Recovery ¹	100.5	115.9	104.6	139.2	164.2	102.1	163.4	156.8	166.5	143.1	136 (20.1)
HPC 117 (ng/mL)	Mean absorbance*	1.03770	1.22150	0.84285	1.80205	1.93285	1.32450	1.71665	1.75220	1.85450	1.72615	1.52 (25.1)
	% Recovery ²	108.3	127.5	88.0	188.1	201.8	138.3	179.2	182.9	193.6	180.2	159 (25.1)
Plate Specific CP = 0.16443 (HuSV11RR)												
[†] Mean absorbance value had a CV% that was greater than 20%												
Mean Plate LPC absorbance (in negative control serum) = 0.201												
Mean Plate HPC absorbance (in negative control serum) = 0.958												
*Mean absorbance is mean of duplicates												
¹ % Recovery = 100*Mean absorbance /Mean Plate LPC absorbance												
² % Recovery = 100*Mean absorbance /Mean Plate HPC absorbance												
Mean and %CV data rounded to 3 significant figures												

Conclusion appendix 1:

The sponsor adequately responded to our information requests dated Oct 22, 2018. No further information are needed.

APPENDIX 2:

Following comments were sent to sponsor on June 19, 2020:

1. Your amended final assay validation report submitted to the NDA application on March 27, 2020 indicates that anti-glucagon antibody assay validity may be affected by matrix interference at the levels of LPC and HPC concentrations of positive control antibodies. While the signals produced by LPC and HPC spiked into each of 10 individual serum samples were above the plate-specific cut-point for that assay, the recovery (% recovery) of most of these samples is unacceptably high. These results suggest that the signals produced in these samples may not reliably support the lack of matrix interference in this assay. Matrix interference, however, was not seen during validation of the anti-ZP4207 antibody binding assay that uses the same positive control antibody. Explain this observation and provide data justifying the acceptability of the observed matrix effect results in the anti-glucagon antibody binding assay.
2. Assay method standard operating procedure (SOP) summaries are included in your assay validation reports. However, full method SOPs including assay plate layouts that demonstrate inclusion of additional sensitivity controls in each assay plate, as requested by the Agency, were not provided. Provide full method SOPs for each immunogenicity assay method. The SOPs may be included in Module 1.

Responses to question 1:

The sponsor stated that the anti-glucagon and anti-ZP4207 (dasiglucagon) antibody assays were designed to be as similar as possible and shared a positive control antibody. However, to ensure relevant epitope representation in the respective assays, either dasiglucagon or glucagon was used (b) (4). The sequence homology between dasiglucagon and glucagon is (b) (4) not 100% identical which could explain the difference between the signal levels of the two assays.

Based on mean absorbance, a number of the spiked individual matrix effect samples in the anti-glucagon assay resulted in higher recoveries than expected. So, they repeated the experiment twice, resulting in average recoveries of 136% and 159% relative to the plate LPC and HPC, respectively (Study 14-174, Table 16). They noted that despite the high recovery in individual matrix samples for all three repeat assay runs, the matrix effect evaluation met the acceptance criteria which required all spiked samples to be above the plate specific cut point and thereby be identified as "screened positive". This acceptance criteria is in line with current thinking

concerning selectivity evaluation of antibody screening assays where 8 or 9 out of 10 selectivity samples score as ADA positive¹⁴. The sponsor stated that as they observed amplification of the signal, it potentially results in an improved sensitivity and the risk of false negative results is further reduced. In conclusion, the selectivity was considered acceptable for the intended purpose of the assay.

Reviewer comment: The sponsor suggested that the sequence homology between dasiglucagon and glucagon may have caused the difference between the signal level of the two assays. However, this difference in response signal between assays does not explain how the recovery in the anti-glucagon antibody assay was relatively higher in 6 of 10 samples for LPC and 7 of 10 samples for HPC samples when compared with the recoveries in anti-dasiglucagon antibody assay. As a result, it is assumed that the matrix effect or interference due to matrix component may exist in these samples.

The matrix interference on an analytical method is expected to cause by other components of a sample except the compound which is being tested. The data presented in all 3 experiments showed that all samples prepared without a positive control antibody had always a response below the plate specific cut point whereas, all samples prepared with positive control antibody had a response above the plate specific cut point as expected. However, the recovery in these assay runs resulted to be higher than expected. Although the sponsor's response did not address this issue directly, in my opinion the observation may have been caused due to the presence of an unknown component in the reaction mix which may not be a matrix component. Nevertheless, false-positive reporting (as opposed to a false-negative reporting) using matrix samples spiked with assay PC material, is typically not a safety or regulatory concern and all positive controls had response higher than cut-point as expected and required by the assay acceptance criteria, therefore sponsor's response is acceptable.

Responses to question 2:

The sponsor stated that detailed assay methods used in the assay validations related to binding antibodies were provided with the validation reports in Module 5.3.1.4 with the original NDA on 27 March 2020. An overview of where to find these assay method descriptions is provided in Table 1 (did not copy in this memo). For neutralizing antibodies, the Sponsor provided the protocols and protocol amendments for the validation method together with this response. Links are also provided in Table 2 (did not copy in this memo). Antibody analysis reports for each conducted clinical trial were submitted with the original NDA. Links are provided to the detailed assay descriptions for each of these antibody reports in Table 3 (did not copy in this memo). IgG and IgM sensitivity control samples were included in all clinical sample analyses initiated after implementation and validation of the assays as requested by the Agency (13-Mar-2018, IND 127866 (b) (4)). In addition to links to the assay descriptions for the

¹⁴ Gorovits B, Roldan MA, Baltrukonis D, Cai CH, Donley J, Jani D, Kamerud J, McCush F, Thomas JS, Wang Y. Anti-drug Antibody Assay Validation: Improved Reporting of the Assay Selectivity via Simpler Positive Control Recovery Data Analysis. AAPS J. 2019;21(5):76.

antibody analysis reports for each conducted clinical trial, Table 3 also includes information on whether the IgG and IgM sensitivity controls had been implemented at the time of the analysis.

Reviewer's comment: The responses are adequate.

Conclusion appendix 2:

The sponsor adequately responded to our information requests dated June 19, 2020. No additional information are needed.

APPENDIX 3:

FDA IR Comment to sponsor dated 10/09/2020:

The 'Integrated summary of Immunogenicity' (ISI: page 52-53) states that you analyzed the clinical samples from studies ZP4207-14013, ZP4207-15007, ZP4207-15126, ZP4207-16051, ZP4207-16098 and ZP4207-16136 initially using the validated confirmatory cut-points (CCP) determined at 0.1% false positive rate (FPR). Later you re-evaluated the samples from these studies using a CCP determined at 1% FPR, based on the recommendation provided in the FDA immunogenicity guidance⁴. However, data from this revised evaluation were not found in your submission. The footnotes of the tables for the confirmatory assay results provided in the analytical reports for these individual studies (for example, Tables 9-13, 23-28, 30 and 33 from study ZP4207-14013; or Tables 2, and 5 from study ZP4207-16098) indicate that the analysis was carried out based on CCPs of 0.51109 for the anti-ZP4207 assay and 0.76727 for the anti-glucagon assay. These CCP were determined during validation of these assays using the 0.1% FPR. Provide the location of the revised confirmatory assessment results (at 1% FPR) from these trials in your submission or submit the requested data from these trials determined using the revised, 1% FPR-based CCP to the Agency within one week of receiving this letter to allow our continued assessment of your application.

Zealand response:

Zealand Pharma acknowledges that it was not clear in the ISI report how the re-evaluation of ADA data was performed. Zealand confirmed that they used the validated CCP determined at a 0.1% FPR initially in the above-listed early clinical trials, but later they re-evaluated the assay results using the a CCP determined at 1% FPR. However, they did not generate any additional outputs for the bioanalytical reports or the clinical trial reports for the re-evaluation and the reports were not updated in ISI. The ratios of absorbance values (confirmatory) for each of the above-listed trials were manually re-evaluated using the revised CCPs of 0.612478 for dasiglucagon and 0.821605 for glucagon, which were determined at a 1% FPR. An overview of the final confirmatory ADA assay results are provided below in a new table 22 (from sponsor Table 1, Responses to FDA IR dated 09-Oct-2020 to ADA cut-point; SDN 30).

Table 22: Re-evaluation of ADA data based on revised CCP determined at a 1% FPR

Trial	Bioanalytical report ZP study ID/ (b) (4) study ID	Interpretation upon re-evaluation of CCPs		Comments
		Not changed	Changed	
14013 Part 1 (healthy subjects)	14-013/ (b) (4) 040	Tables 9, 11–13 and 23-28	Table 10: Subject (b) (6) Visit 4 anti-dasiglucagon antibody positive	Patient (b) (6) described in ISI Table 6-5 ^a . No treatment boosting as the signal at Visit 4 is lower than the pre-dose signal
14013 Part 2 (T1DM)	14-013/ (b) (4) 040	Table 30	Table 33: Patient (b) (6) Visit 4 anti-glucagon antibody positive	Patient (b) (6) described in ISI Table 6-5 ^b . No treatment boosting in anti-dasiglucagon antibodies. Therefore, not further discussed in ISI.
15007	15-007/ (b) (4) 048	All samples were ADA-negative in screening assay; no confirmatory testing done	None	
15126	16-033/ (b) (4) 065	Tables 5, 7, 8, 13–15	Table 6: Patient (b) (6) Visit 3 and 4 anti-dasiglucagon antibody positive	Patient is discussed in ISI section 4.5.3.1. ^c
16051	16-078/ (b) (4) 072	Tables 2 and 4	None	
16098	16-098/ (b) (4) 076	Tables 2 and 5	None	
16136	16-136/ (b) (4) 081	Tables 3 and 5	Table 2: Patient (b) (6) visit2pd (pre-dose), anti-glucagon antibody positive	No treatment boosting. All post-dose samples were ADA-negative. Therefore, not described in ISI.

Footnotes: a: This patient (screening number (b) (6)) received dasiglucagon 0.1 mg SC and was ADA-positive at pre-dose of Visit 2 (Titer 11.4; Mean OD 0.08770) and at Visit 4 (Follow-up Visit; Mean OD 0.08245).

b: This patient (screening number 092) received GlucaGen 1 mg IM at Visit 2 and dasiglucagon 0.7 mg IM at Visit 3. The patient was ADA-positive for dasiglucagon at pre-dose of Visit 2 (titer: 16.2), at Visit 3 (titer: 22.0) and at Visit 4 (Follow-up Visit; titer: 15.2) and ADA-positive for glucagon at Visit 4 (Follow-up Visit).

c: This patient (screening number 033) received dasiglucagon 0.3 mg SC at Visit 2 and GlucaGen 0.5 mg SC at Visit 3. The patient was ADA-negative at pre-dose of Visit 2 and ADA-positive for dasiglucagon at Visit 3 (Day 7; titer ~22) and Visit 4 (Follow-up Visit; Day 28; titer ~22).

d: This patient received GlucaGen 1 mg SC.

Abbreviations: ADA: anti-drug antibody; CCP: confirmatory cut-point; FPR: false positive rate;

IM: intramuscular(ly); ISI: Integrated Summary of Immunogenicity; OD: optical density; SC: subcutaneous(ly);

T1DM: type 1 diabetes mellitus; (b) (4) ZP: Zealand Pharma.

Reviewer assessment: The sponsor's response to our IR is satisfactory. Changes in treatment due to re-calculation of CCP at 1.0% FPR are minimal, including only one subject with treatment-induced ADA that was previously not captured using CCP defining a 0.1% FPR. This is subject (b) (6) from study 15126. Other subjects affected by CCP modification included pre-dose samples

becoming ADA+ without subsequent boosting after drug treatment, or a post-dose sample from Subject (b) (6) which became reactive against glucagon but did not amplify dasiglucagon ADA titer. No further comment is needed.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

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PATRICK J LYNCH
12/04/2020 02:31:17 PM

Clinical Inspection Summary

Date	11/23/2020
From	Cynthia F. Kleppinger, M.D., Senior Medical Officer Min Lu, M.D., M.P.H., Team Leader Kassa Ayalew, M.D., M.P.H., Branch Chief Good Clinical Practice Assessment Branch (GCPAB) Division of Clinical Compliance Evaluation (DCCE) Office of Scientific Investigations (OSI)
To	Mahtab Niyiyati, M.D., Medical Officer Mitra Rauschecker, M.D., Clinical Team Leader Division of Diabetes, Lipid Disorders, and Obesity (DDLO) Elizabeth Godwin, MSHS, CCRP, Regulatory Project Manager Division of Regulatory Operations for Cardiology, Hematology, Endocrinology, and Nephrology
NDA	214231
Applicant	Zealand Pharma A/S
Drug	dasiglucagon
NME	Yes
Therapeutic Classification	Antihypoglycemic agent
Proposed Indication	Treatment of severe hypoglycemia in patients with diabetes ages 6 years and above
Consultation Request Date	5/12/2020
Summary Goal Date	11/27/2020 (moved up from 2/5/2021)
Action Goal Date	3/26/2021
PDUFA Date	3/27/2021

I. OVERALL ASSESSMENT OF FINDINGS AND RECOMMENDATIONS

The inspection for this new drug application (NDA) consisted of two domestic sites (representing three clinical study sites) in addition to the sponsor.

The COVID-19 global pandemic has significantly limited the Office of Regulatory Affairs' ability to conduct foreign onsite good clinical practice (GCP) inspections. As a result, a remote regulatory assessment of Zealand Pharma source records was performed for the sponsor site located in Denmark.

In general, based on the inspections of the two domestic clinical sites and the remote regulatory assessment of the sponsor foreign site, the inspectional findings support validity of data as reported by the sponsor under this NDA.

II. BACKGROUND

Zealand Pharma A/S has submitted an original new drug application (NDA) for dasiglucagon (ZP4207) injection 0.6 mg/mL, intended for the treatment of severe hypoglycemia in patients with diabetes ages six years and above. Dasiglucagon is a physically and chemically stable peptide analog of human glucagon but with 7 amino acid substitutions, which improve its physical and chemical stability in aqueous media. Studies were conducted under IND 127886.

Inspections were requested for the three pivotal studies ZP4207-16137, ZP4207-17145, and ZP4207-17086.

ZP4207-16137 *A phase 3, randomized, double-blind, parallel trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo and with reference to GlucaGen®*

This was a global, multicenter, randomized, parallel, and double-blind clinical trial confirming the efficacy and safety of dasiglucagon for insulin-induced hypoglycemia in adult subjects with type 1 diabetes mellitus (T1DM).

The first subject was enrolled [REDACTED] (b) (6) and the trial completed May 25, 2018. There were five trial centers: two in Germany and one each in Austria, Canada and the United States of America (USA).

There were 235 subjects screened and 170 subjects were randomized. One hundred and sixty-eight (168) subjects received trial drug and all dosed subjects completed the trial. Two subjects in the dasiglucagon group discontinued prior to being dosed.

The primary endpoint is time to plasma glucose recovery. Plasma glucose recovery was defined as first increase in plasma glucose of ≥ 20 mg/dL (1.1 mmol/L) from baseline without administration of rescue intravenous (IV) glucose. Samples were collected pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 50, 60, 75 and 90 minutes after dosing.

ZP4207-17145 *A randomized, double-blind, parallel-group trial to confirm the clinical efficacy and safety of dasiglucagon in the rescue treatment of hypoglycemia in subjects with type 1 diabetes mellitus (T1DM) compared to placebo*

This was a multi-center, randomized, parallel-group, double-blind study conducted in adult subjects with T1DM at 3 centers in the USA.

The first subject enrolled [REDACTED] (b) (6) and the last subject visit was [REDACTED] (b) (6). The study screened 68 subjects and 45 subjects were randomized to either dasiglucagon (34 subjects) or placebo (11 subjects); 44 subjects completed the study (i.e., completed the 28-day follow-up Visit 3 or the last scheduled procedure). One subject randomized to placebo withdrew consent prior to dosing.

The primary endpoint was time to plasma glucose recovery. Plasma glucose recovery is defined as

first increase in plasma glucose of ≥ 20 mg/dL (1.1 mmol/L) from baseline without administration of rescue IV glucose. Samples were collected pre-dose, and at 4, 6, 8, 10, 12, 15, 17, 20, 25, 30, 40, 45, 50, 60, 75 and 90 minutes after dosing.

ZP4207-17086 *A phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm trial to assess the efficacy, safety, and pharmacokinetics of dasiglucagon relative to placebo and GlucaGen® when administered as a rescue therapy for severe hypoglycemia in children with T1DM treated with insulin*

This was a randomized, double-blind, placebo- and active-controlled, parallel-group study at five sites in the USA (3), Slovenia (1), and Germany (1). This trial aimed to evaluate the safety and efficacy of dasiglucagon by subcutaneous (SC) administration in pediatric T1DM patients. The trial further aimed to evaluate the pharmacodynamics (PD) and pharmacokinetics (PK) of dasiglucagon. The trial recruited male and female patients (≥ 6 years and <18 years of age, body weight ≥ 20 kg) with T1DM for at least 1 year.

The first subject was enrolled [REDACTED] (b) (6) and the last subject visit was [REDACTED] (b) (6).

There were 59 subjects screened, 42 subjects randomized, and 41 subjects who completed the trial. One subject withdrew after randomization but before treatment.

The primary endpoint was time to plasma glucose recovery, defined as the first increase in plasma glucose of ≥ 20 mg/dL (1.1 mmol/L) from baseline without administration of rescue IV glucose. After the start of insulin infusion, plasma glucose had to be checked approximately every 10 minutes while plasma glucose was above 110 mg/dL and approximately every 5 minutes once plasma glucose was at or below 110 mg/dL and until the time point after dosing where the patient's plasma glucose had returned to ≥ 70 mg/dL (3.9 mmol/L).

Dasiglucagon and placebo were available as a liquid formulation and GlucaGen® as a powder for reconstitution. Since the trial medications were not identical in appearance, the handling, preparation and administration of trial medication was to be done by unblinded trial personnel who were not to be involved in any other trial procedures or assessments.

III. RESULTS (by Site)

NOTE: Site inspections focused on review of informed consent documents (ICDs), institutional review board (IRB)/ ethics committee (EC) correspondences, 1572s/investigator agreements, financial disclosures, training records, CVs and licenses, delegation of duties, monitoring logs and reports, inclusion/exclusion criteria, enrollment logs, subject source documents including medical history records, drug accountability, concomitant medication records, and adverse event reports. Source records were compared to the sponsor's data line listings.

1. Timothy S. Bailey, M.D.
AMCR Institute
625 West Citracado Parkway, Suite 112
Escondido, CA 92025-6428

Site: 005 Study Number: ZP4207-17086

Dates of inspection: October 6 – 13, 2020

There were 36 subjects screened and 23 subjects enrolled into the study; 23 subjects completed the study. There were 36 subject records reviewed.

Dr. Bailey is a board-certified endocrinologist and CEO/President of Advanced Metabolic Care + Research (AMCR) Institute. He has been doing clinical research since 1990.

The institutional review board (IRB) of record was the study's central IRB, (b) (4).

Source records were available and organized. Records did not completely, accurately and consistently document the staff member who administered the investigational product.

Source records were compared to the sponsor data line listings. There was no under-reporting of adverse events. All onsite plasma glucose concentrations measured using the glucose analyzer YSI 2300 taken for safety were reviewed and no deviations were noted. Endpoint data were not available at the site as endpoint plasma glucose measurements were sent to the central laboratory for processing and not returned to the site. The FDA inspector cross-referenced times that blood draws were taken for the central lab with the plasma glucose times available in the sponsor data line listings and did not find any discrepancies.

During the inspection, it was discovered that Dr. Bailey phone-consented subjects' parents without IRB approval and did not follow IRB instruction to obtain consent from both parents. In cases where the second parent did not give consent prior to screening procedures, no reason was documented.

At the conclusion of the inspection, a Form FDA-483, Inspectional Observations, was issued for not always obtaining the permission of both parents as per 21CFR 50.53. Twenty of the 36 screened subjects did not have parental consent from both parents prior to screening procedures.

OSI Reviewer Comment: Although regulatory violations were noted as described above, Dr. Bailey did not conduct any high-risk procedures or any dosing of investigational product prior to getting consent from each parent. All subjects that underwent dosing at Visit 2 did have the consent of both parents prior to that procedure. The violation should not impact safety and efficacy analyses. Dr. Bailey's October 26, 2020 response to the observations was reviewed and deemed acceptable.

2. Julie A. Willard, M.D.
Attention: Bridgette B. Franey, M.D.
855 3rd Avenue, Suite 4400
Chula Vista, CA 91911

Site Number: 14 **Study Number:** ZP4207-16137

Site Number: 101 **Study Number:** ZP4207-17145

Dates of inspection: October 13 – 16, 2020

For Study ZP4207-16137, there were 37 subjects screened and 24 subjects enrolled into the study; 24 subjects completed the study. There were 37 subject records reviewed.

For Study ZP4207-17145, there were 37 subjects screened and 21 subjects enrolled into the study; 20 subjects completed the study. One subject (Subject (b)(6)) randomized to placebo did not wish to continue with the study and voluntarily withdrew consent from the study on Day 1 prior to investigational product administration. There were 37 subject records reviewed.

ProSciento, Inc. is a specialty contract research organization (CRO) that has been in business since 2003. Dr. Julie Willard was the original investigator hired by the CRO before leaving 12/13/2019 due to reasons unrelated to the studies. Dr. Robert Peters, M.D., PhD was a sub-investigator for most of the study; however, he took over as site investigator from 3/10/2018 through 7/17/2018 when Dr. Willard was unavailable. Dr. Bridgette Franey took over responsibility as site investigator for both studies, although the two studies were already closed.

It was noted that the study personnel listed as sub-investigators on the Site Signature/Delegation Log for Site 14 should have been listed on the Form FDA 1572s and were required to complete financial disclosure forms and submit them to the sponsor.

The institutional review board of record for both studies was the central IRB, (b)(4).

Source records were compared to the sponsor data line listings. There was no under-reporting of adverse events. All onsite plasma glucose concentrations measured using the glucose analyzer YSI 2300 taken for safety were reviewed and no deviations were noted. Endpoint data were not available at the site as endpoint plasma glucose measurements were sent to the central laboratory for processing and not returned to the site. The FDA inspector cross-referenced times that blood draws were taken for the central lab with the plasma glucose times available in the sponsor data line listings, in addition to shipping records to the central laboratory and did not find any discrepancies.

The inspection revealed adequate adherence to the regulations and the investigational plan. There were no objectionable conditions noted and no Form FDA-483, Inspectional Observations, issued.

3. Zealand Pharma A/S
Sydmarken 11
2860 Søborg
Denmark

Dates of inspection: June 15 – 24, 2020

Due to the COVID-19 pandemic, travel to the sponsor site was not possible. Therefore, FDA's Office of Regulatory Affairs (ORA) conducted a desktop assessment of Studies ZP4207-16137, ZP4207-17145, and ZP4207-17086. Video conferencing via WebEx, document sharing via an online platform (box.com), and read-only access to the online trial master file (TMF) information were utilized to exchange information. The FDA inspectors' review was significantly limited in scope due to the logistical constraints of this information exchange.

The audit included reviewing the organizational structure and responsibilities, transfer of obligations, vendor contracts, safety reporting, site and monitor selections, monitoring, quality assurance, randomization, test article handling, protocol deviations, 1572s and investigator agreements, financial disclosures, study registration on ClinicalTrials.gov, training, and electronic data systems.

Records for all three studies were available during the inspection. Records were either kept electronically in a TMF or were paper-based.

Zealand Pharma was founded in 1998 in Denmark and there are currently three additional locations in the US. The company moved into their current location in September 2019.

There were no investigators terminated from the studies and no issues with noncompliant investigators. Monitoring procedures and activities appeared comprehensive and adequate. The monitoring plans visit schedules were not always followed by the contracted monitors.

The investigator (Julie Willard) at Site 14 for Study ZP4207-16137 left the site December 2019. A memorandum dated 6/16/2020 from Site 14 explained that Dr. Bridgette Franey, M.D. would represent the site in an investigator capacity for previously conducted trials when the investigator is no longer employed at the site.

It was also noted that the study personnel listed as sub-investigators on the Site Signature/ Delegation Log for Site 14 should have been listed on the Form FDA 1572s and were required to complete financial disclosure forms and submit them to the sponsor. A Site 14 Note-To-File dated 10/2/2018 explained their reasons for not including some sub-investigators on the Form FDA 1572. Sponsor staff said they felt the reasoning was acceptable.

For the Slovenian site in Study ZP4207-17086, monitoring reports discussed the

investigational product being stored in a shared refrigerator with access to all study staff, including blinded staff. Sponsor staff stated that there was no ability to unblind. A refrigerator and a lockbox were then ordered for the site in January 2019. No other objections were noted.

Study ZP4207-16137 Site 13 in Austria had 13 protocol deviations for increasing the insulin infusion rate above 200% of the basal rate. Protocol version 5.0 added two words to read, “0 to 200% or more,” and was approved at Site 13 by the ethics committee on 5/9/2018. By 5/9/2018, all subjects at this site had completed their treatment. This protocol deviation was not found for any other sites. On 3/27/2018, the CRO Sr. Clinical Team Lead wrote sponsor staff to question this recurring protocol deviation, which was brought to their attention by Site 12 in Mainz, Germany. Sponsor staff told the CRO that “If 0-200% of the basal rate is not sufficient, this can be deviated from at the investigator’s discretion.” This change to the protocol was allowed without prior written ethics committee approval, which occurred 5/9/2018.

Regarding Study ZP4207-16137, the Clinical Study Report (CSR) section 16.1.4 did not include sub-investigators for Site 14, did not include the two subjects randomized but not treated in the table column “Number of Subjects Randomized”, and incorrectly stated the Coordinating Investigator as Thomas Pieber instead of Ulrike Hoevelmann. Dr. Pieber was never the Coordinating Investigator. Sponsor staff said these errors will be corrected and submitted with other corrections to the CSR.

Deviations were discussed at the final conference call with the firm. They included ensuring all sub-investigators are listed on the FDA 1572 and are signing financial disclosure forms, ensuring the monitoring plans are followed, and ensuring protocol deviations are properly recorded and addressed.

The inspection revealed adequate adherence to the regulations and the investigational plan. There were no objectionable conditions noted and no Form FDA-483, Inspectional Observations, issued.

Data from this sponsor appear acceptable.

{See appended electronic signature page}

Cynthia F. Kleppinger, M.D.
Senior Medical Officer
Good Clinical Practice Assessment Branch
Division of Clinical Compliance Evaluation
Office of Scientific Investigations

CONCURRENCE: *{See appended electronic signature page}*

Cynthia F. Kleppinger, M.D. signing for
Min Lu, M.D., M.P.H.
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Kassa Ayalew, M.D., M.P.H
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cc:

Central Doc. Rm./ NDA 214231
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DDLO /Acting Deputy Director/ Patrick Archdeacon
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DDLO /Clinical Reviewer/ Mahtab Niyiyati
DRO /Regulatory Project Manager/Elizabeth Godwin
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/s/

CYNTHIA F KLEPPINGER

11/23/2020 03:34:46 PM

Acting Team Leader. Signature covering for Min Lu, MD, MPH, Team Leader.

KASSA AYALEW

11/23/2020 04:54:10 PM



DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

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Division of Pediatrics and Maternal Health Review

Date: November 16, 2020 **Date consulted:** 4/13/2020

From: Christos Mastroyannis, M.D., Medical Officer, Maternal Health,
Division of Pediatrics and Maternal Health (DPMH)

Through: Tamara Johnson, MD, MS, Team Leader, Maternal Health, DPMH

To: Division of Diabetes, Lipid Disorders, and Obesity (DDLO)

Drug: Dasiglucagon injection, for subcutaneous (SC) use. An aqueous
formulation for a single-dose injection.

NDA: 214231

Applicant: Zealand Pharma A/S

Subject: Pregnancy and Lactation Labeling Formatting Recommendations

Indication: For the treatment of severe hypoglycemia

Materials Reviewed:

- March 27, 2020 Original Submission for NDA 214231, a New Molecular Entity (NME)
- April 13, 2020, DDLO consult, DARRTS Reference ID 4591439

Consult Question: “Review of the FPI for PLLR compliance, and any additional labeling recommendations from the Maternal Health Team to ensure the safe use of dasiglucagon in patients of childbearing potential”.

INTRODUCTION AND BACKGROUND

On March 27, 2020, Zealand Pharma A/S, submitted an original NDA 214231 for dasiglucagon

for subcutaneous (SC) injection under the 505 (b)(1) pathway for the treatment of severe hypoglycemia. With this submission, the applicant provided labeling to comply with PLLR. The Division of Diabetes, Lipid Disorders, and Obesity (DDLO) consulted the Division of Pediatrics and Maternal Health (DPMH) on April 13, 2020, to assist with the Pregnancy and Lactation subsections of labeling.

Dasiglucagon Drug Characteristics¹

- Dasiglucagon is a physically and chemically stable peptide analog of human glucagon.
- Like glucagon, dasiglucagon is comprised of 29 amino acids, but 7 amino acid substitutions have been introduced to improve physical and chemical stability in aqueous media.
- It is a ready-to-use aqueous formulation in a
 - pre-filled safety syringe or
 - auto-injector (HypoPal)

Table 1: Dasiglucagon Drug Characteristics

Drug Class	Antihypoglycemic
Mechanism of Action	Dasiglucagon is a specific glucagon receptor agonist, which increases blood glucose concentration by activating hepatic glucagon receptors, thereby stimulating glycogen breakdown and release of glucose from the liver. Hepatic stores of glycogen are necessary for dasiglucagon to produce an anti-hypoglycemic effect.
Molecular Weight	3,382 g/mol
Terminal Half-Life	30 min
Volume distribution following IV administration.	Approximately 30% of the body weight, indicating that dasiglucagon is distributed in the extracellular space
Protein Binding	A reliable protein-binding value would not be obtainable because the measurement needs to be conducted at 37°C, a condition in which dasiglucagon is degraded by proteases.

REVIEW

PREGNANCY

Hypoglycemia and Pregnancy

According to a publication by Kimmerle *et al.* 1992,² severe hypoglycemia (SH) [defined as impairment of consciousness due to hypoglycemia subsequently treated with glucagon or intravenous glucose] is common during pregnancies in Type I diabetic women. Pregnant women with type 1 diabetes have three to five times more hypoglycemic episodes than during the period prior to pregnancy.³ Kimmerle *et al.* followed 77 women with Type I diabetes, who had 85 pregnancies, from 1986-1990. There were 94 episodes of SH reported during 35 of these pregnancies. Of 94 episodes of SH, 84% occurred before the 20th gestational week (median 12th week) and 77% during sleep. In the group with SH, there was no permanent

¹ Applicant’s dasiglucagon Product Insert/Labeling of March 27, 2020

² Kimmerle R et al. Severe Hypoglycemia Incidence and Predisposing Factors in 85 Pregnancies of Type I Diabetic Women. Diabetes Care.1992; 15(8):1034-7.

³ Ringholm L, Pedersen U: Hypoglycemia during pregnancy in women with type 1 diabetes. Diabet Med 29: 558, 2012.

maternal sequelae, and there was a favorable fetal outcome (no perinatal death and no congenital malformation). Mean HbA_{1c} values were not different between the group with and without SH for the first half (6.4 ± 1.1 vs. $6.3 \pm 0.9\%$) and 2nd half (5.4 ± 0.6 vs. $5.5 \pm 0.7\%$) of pregnancy.²

Risk factors for severe hypoglycemia during pregnancy include a history of severe hypoglycemia in the year preceding pregnancy, impaired hypoglycemia awareness, long duration of diabetes, low hemoglobin A_{1c} in early pregnancy, fluctuating plasma glucose levels, and excessive use of insulin injections between meals.^{1,4} Hypoglycemia generally presents as sweating, tremors, blurred or double vision, weakness, hunger, confusion, paresthesias, anxiety, palpitations, nausea, headache, or stupor. Hypoglycemia may even cause death.⁵ Moderate and infrequent hypoglycemic episodes are generally well tolerated by the fetus.⁶ With the increasing duration of pregnancy, fetal glucose consumption increases and there is a risk of maternal, and possibly fetal, hypoglycemia, particularly if there are long intervals between meals or at night. Pregnant women with diabetes, especially Type 1 diabetes, should be educated about the symptoms and treatment of hypoglycemia.

Mild hypoglycemia (i.e., a glucose level of <70 milligrams/dL [3.89 mmol/L] in patients who are able to follow commands) should be treated by giving juice, glucose, or food by mouth. Standard treatment for more severe hypoglycemia, include IV glucose or PO glucose or glucagon.⁷

Applicant's Review

Nonclinical Data

In reproduction studies, daily subcutaneous administration of dasiglucagon to pregnant rabbits and rats during organogenesis did not cause adverse developmental effects at maternal exposures 7 and 703 times the human dose of 0.6 mg, based on AUC. In rabbits, visceral/skeletal abnormalities were seen at repeated high exposures (20 and 99 times the human dose, based on AUC), which are considered secondary to repeated maternal hyperglycemia. As per applicant, for use of dasiglucagon as a single dose rescue treatment for severe hypoglycemia, “the risk is considered minimal” because of the short duration of treatment and short duration of the blood glucose increase.⁸

Human Data

The applicant reports that there is no relevant human literature. The drug has not been used in pregnant women. Females who were pregnant (according to a positive pregnancy test), or were actively attempting to get pregnant, or were lactating, were to be excluded from participation in all clinical trials of dasiglucagon.

⁴ Obstetrics, 24e <https://accessmedicine.mhmedical.com/book.aspx?bookid=1057>

⁵ Leinonen PJ, Hiilesmaa VK, Kaaja RJ: Maternal mortality in type 1 diabetes. *Diabetes Care* 24(8):1501, 2001

⁶ Lee-Parritz A: New technologies for the management of pregestational diabetes. *Obstet Gynecol Surv* 67: 167, 2012

⁷ Uptodate. Cryer PE. Hypoglycemia in adults with diabetes mellitus. Last updated: September 16, 2019

⁸ From applicant's submission of March 27, 2020 (2.4)

Pharmacovigilance Review

The applicant reports only one pregnancy during the drug development program in clinical trials, however, not in the dasiglucagon group. A 32-year-old female patient in trial 16136 became pregnant approximately 2 months after the last administration of investigational product. The pregnancy was uneventful. The baby was delivered by cesarean section 5.5 weeks before term due to an abnormal heart rate. The baby was healthy.

Drug Utilization

This is a new drug product that has not been approved yet. Therefore, there is no drug utilization to be reported.

DPMH's Review of Literature

DPMH conducted a search of published literature using PubMed and Embase regarding dasiglucagon exposure during pregnancy. No publications were identified. Reprotox⁹, in regard to glucagon, states that:

Use of glucagon during human pregnancy was described in 7 insulin-dependent diabetics in response to insulin reactions.¹⁰ Although adverse effects attributed to glucagon were not described, little can be concluded from this report with regard to fetal safety.

GG Briggs and RK Freeman in Drugs in Pregnancy and Lactation: A Reference Guide to Fetal and Neonatal Risk does not have an entry.

Review of glucagon by DPMH¹¹ based on case reports and a small number of observational studies with glucagon use in pregnant women concluded that, over decades of use, no drug-associated risk of major birth defects, miscarriage or adverse maternal or fetal outcomes have been identified.

Summary

There are no data of use of dasiglucagon in pregnant women. Like glucagon, dasiglucagon is comprised of 29 amino acids, but 7 amino acid substitutions have been introduced to improve physical and chemical stability in aqueous media. Severe hypoglycemia is a medical emergency. Dasiglucagon treatment includes only one dose. When the patient responds to treatment, he/she should start on oral carbohydrates. Because of one dose administration, the available clinical data on glucagon, and no evidence of teratogenicity in animal studies, it appears that the risk to the fetus is low. In a risk/benefit analysis, the fetus would be in a worse condition if severe hypoglycemia persists. Therefore, dasiglucagon should not be withheld from the pregnant woman due to concerns about its effects on the developing fetus. Prolonged experience over several decades with use of glucagon based on published observational studies and postmarketing reports have not identified a drug-associated risk of major birth defects, miscarriage or adverse maternal or fetal outcomes. There are risks associated with untreated severe hypoglycemia in pregnancy.

⁹ Truven Health Analytics LLC. Micromedex.

¹⁰ Rayburn W, Piehl E, Sanfield J, Compton A: Reversing severe hypoglycemia during pregnancy with glucagon therapy. *Am J Perinatol* 1987;4:259-61

¹¹ Labeling review for Glucagon by Jane E Liedtka, M.D., in DARRTS dated 4/12/2019 and Reference ID: 4417323

LACTATION

Nonclinical Data

There is no information available on the presence of dasiglucagon in animal milk, or the effects of the drug on milk production.

Human Data

The applicant did not perform a search for publications related to lactation and associated with dasiglucagon. Dasiglucagon is an NME. DPMH searched LactMed, Micromedex, and of published literature in PubMed using the search terms “dasiglucagon” and “lactation”, and/or “breastfeeding.” No published studies of dasiglucagon use in lactating women were found, nor any published case reports. There is no information available on the presence of dasiglucagon in human or animal milk, the effects of the drug on the breastfed infant, or the effects of the drug on milk production. GG Briggs and RK Freeman in Drugs in Pregnancy and Lactation: A Reference Guide to Fetal and Neonatal Risk and Hale’s Medications and Mother’s Milk do not have any entries on the drug.

Dasiglucagon, being a peptide, would be unlikely to be absorbed intact from the infant’s GI tract or to have any local effect on the infant’s GI tract. Additionally, dasiglucagon has a short plasma half-life (30 minutes) and high molecular weight, thus limiting amounts available to be present in breast milk and taken by the infant.

FEMALES AND MALES OF REPRODUCTIVE POTENTIAL

Nonclinical Data

In vitro and in vivo genotoxicity studies and a male and female fertility study in rats were conducted. Genotoxicity was evaluated in an Ames test, a chromosome aberration test in human lymphocytes, and an in vivo micronucleus study in rats. These tests revealed no genotoxic potential of dasiglucagon. In a fertility and early embryofetal development study in rats, dasiglucagon administered by subcutaneous injection (0.5, 2, and 8 mg/kg/day) to male and female rats did not impair fertility.

Human Data

Neither the applicant nor this reviewer identified any publications on the effects of dasiglucagon on human fertility for females and males of reproductive potential.

Summary

There is no information to provide to the prescriber regarding pregnancy testing, contraception, or effects on fertility. The drug is to be administered once on an emergency basis for the treatment of severe hypoglycemia. With no demonstration of adverse developmental effects in the animal offspring, pregnancy testing and contraception is unnecessary. Contraception is not needed because its use is an emergency.

DISCUSSION AND CONCLUSIONS

Pregnancy

In animal reproduction studies, no evidence of teratogenic effects is observed in rats and rabbits when dasiglucagon was administered during embryo fetal development. Daily subcutaneous administration of dasiglucagon to pregnant rabbits and rats during organogenesis did not cause adverse developmental effects at maternal exposures 7 and 703 times the human

dose of 0.6 mg, based on AUC. In rabbits, visceral/skeletal abnormalities were seen at repeated high exposures (20 and 99 times the human dose, based on AUC), which are considered secondary to repeated maternal hyperglycemia.

Dasiglucagon does not cross the human placenta. No safety signals were identified from the applicant's pharmacovigilance data in regard to congenital malformations and miscarriages associated with dasiglucagon-exposure during pregnancy. In addition, prolonged experience over several decades with use of glucagon based on published observational studies and postmarketing reports have not identified a drug-associated risk of major birth defects, miscarriage or adverse maternal or fetal outcomes. Therefore, as severe hypoglycemia is a medical emergency, dasiglucagon should not be withheld from the pregnant woman due to concerns about its effects on the developing fetus. Based on the relevant safety experience from glucagon and dasiglucagon's single administration as an emergency treatment, there is no need for a postmarketing pregnancy study.

DPMH recommends the background risk statement in the Risk Summary of subsection 8.1 Pregnancy of the labeling be omitted because dasiglucagon is to be administered once as an emergency treatment for severe hypoglycemia, that is a life-threatening condition.

Lactation

There is no information available on the presence of dasiglucagon in either human or animal milk, the effects of the drug on the breastfed infant, or the effects of the drug on the milk production. The drug being a peptide, will likely be destroyed in the infant's GI tract.

For the same reason mentioned above, there is no need for a postmarketing milk-only clinical lactation study.

DPMH is in agreement with the Division that the risk/benefit summary in the labeling subsection 8.2 Lactation, Risk Summary should be omitted because risk/benefit analysis is concluded when the treatment is administered. Severe hypoglycemia is a life-threatening condition for the mother, and under these circumstances, the mother is not likely to breastfeed. Treatment to the mother should not be withheld for concerns regarding the breastfed infant.

Females and Males of Reproductive Potential

No publications on clinical experience with dasiglucagon and its effects of human fertility were found. Animal reproductive studies of administration of dasiglucagon injection did not show any adverse effects on fertility. DPMH recommends that subsection 8.3 Females and Males of Reproductive Potential is omitted from dasiglucagon labeling.

LABELING RECOMMENDATIONS

DPMH revised subsections 8.1 and 8.2 of dasiglucagon labeling for compliance with the PLLR (see below). Labeling recommendations were discussed with the Division on November 9, 2020. DPMH refers to the final NDA action for final labeling.

DPMH Proposed Pregnancy and Lactation Labeling

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

There are no available data on dasiglucagon use in pregnant women to evaluate for a drug-associated risk of major birth defects, miscarriage or adverse maternal or fetal outcomes. (b) (4)

In animal reproduction studies, daily subcutaneous administration of dasiglucagon to pregnant rabbits and rats during the period of organogenesis did not cause adverse developmental effects at exposures 7 and 70 (b) (4) times the human dose of 0.6 mg based on AUC, respectively (*see Data*). (b) (4)

Data

Animal Data

In an embryo-fetal development study, pregnant rats were treated daily with subcutaneous doses of 2, 10, and 24 mg/kg/day during the period of organogenesis (gestation day 6 to 17). Maternal toxicity, in terms of decreased body weight gain, lower fetal body weight, and delayed bone ossification were observed at ≥ 10 mg/kg/day (≥ 47 (b) (4) times the human dose, based on AUC).

In an embryo-fetal development study, pregnant rabbits were treated daily with subcutaneous doses of 0.1, 0.3, and 1 mg/kg/day during the period of organogenesis (gestation day 6 to 19). Lower fetal body weight and delayed bone ossification were observed at 1 mg/kg/day ((b) (4) times the human dose, based on AUC) a dose that also induced maternal toxicity in terms of decreased body weight gain. At ≥ 0.3 mg/kg/day (20 times the human dose), dasiglucagon caused fetal skeletal and visceral malformations. No adverse fetal developmental effects were observed at 0.1 mg/kg/day, corresponding to exposure 7 times the human dose.

8.2 Lactation

Risk Summary

There is no information on the presence of dasiglucagon in either human or animal milk, the effects of the drug on the breastfed infant, or (b) (4) milk production.

(b) (4) dasiglucagon is a peptide and would be expected to be broken down to its constituent amino acids in the infant's digestive tract and is therefore, unlikely to cause harm to an exposed infant.

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CHRISTOS MASTROYANNIS
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11/16/2020 11:08:24 AM



Center for Drug Evaluation and Research

Division of Cardiovascular and Renal Products

DCN Consult NDA 214231

DATES: Date of Document: 3/27/2020 and 8/31/2020
Date of Consult: 9/2/2020
Date of Assignment: 7/10/2020
Desired Completion Date-1: 10/5/2020
Desired Completion Date-Revised: 10/19/20
Date of Completion: 10/21/2020

FROM: Preston M. Dunnmon, M.D., M.B.A., Medical Officer
Division of Cardiology and Nephrology (DCN)

THROUGH: Norman Stockbridge, M.D., Ph.D.,
Division Director, DCN

TO: Elizabeth R. Godwin
Regulatory Program Manager, DRO-CHEN

DRUG NAME: Dasiglucagon

DOSE/FORMULATION: 0.6 mg as single subcutaneous dose; age $\geq$ 6 years

PRODUCT CLASS: Glucagon receptor agonist

APPLICANT: Zealand Pharma A/S

INDICATION: For treatment of severe hypoglycemia in patients with
diabetes aged 6 years and above

RELATED APPLICATION: IND 127866

DOCUMENTS REVIEWED:

- QT-IRT Consult to IND 127866 dated 2/28/2019 in DARRTS
- DCRP Consult to IND127866 dated 4/17/2019 in DARRTS
- IRT-CS Consult to IND 127866 QT Study Review dated 2/13/2020 in DARRTS
- IRT-CS Consult to NDA 214231 QT Study Review dated 7/28/2020 in DARRTS

EXECUTIVE SUMMARY AND RESPONSES TO CONSULT QUESTIONS

The Review Division requests DCN's opinion regarding PR interval prolongations that have been designated outliers in two subjects from the pooled placebo-controlled studies

DCRP Consult NDA 214231

16167 and 17145 (Subject (b) (6) and Subject (b) (6)), and two subjects receiving a dose at least as high as the intended to-be-marketed dose from a broad pool from all subjects in the development program (subjects (b) (6)). One subject was noted to have transient non-specific ST-T-wave changes post-dose that was reported as an ECG adverse event.

CONSULT QUESTIONS AND DCN REPOSES:

1. Please provide your assessment on whether events of transient PR prolongation are considered clinically meaningful and, if applicable, provide your thinking on whether these events may have future labeling implications.

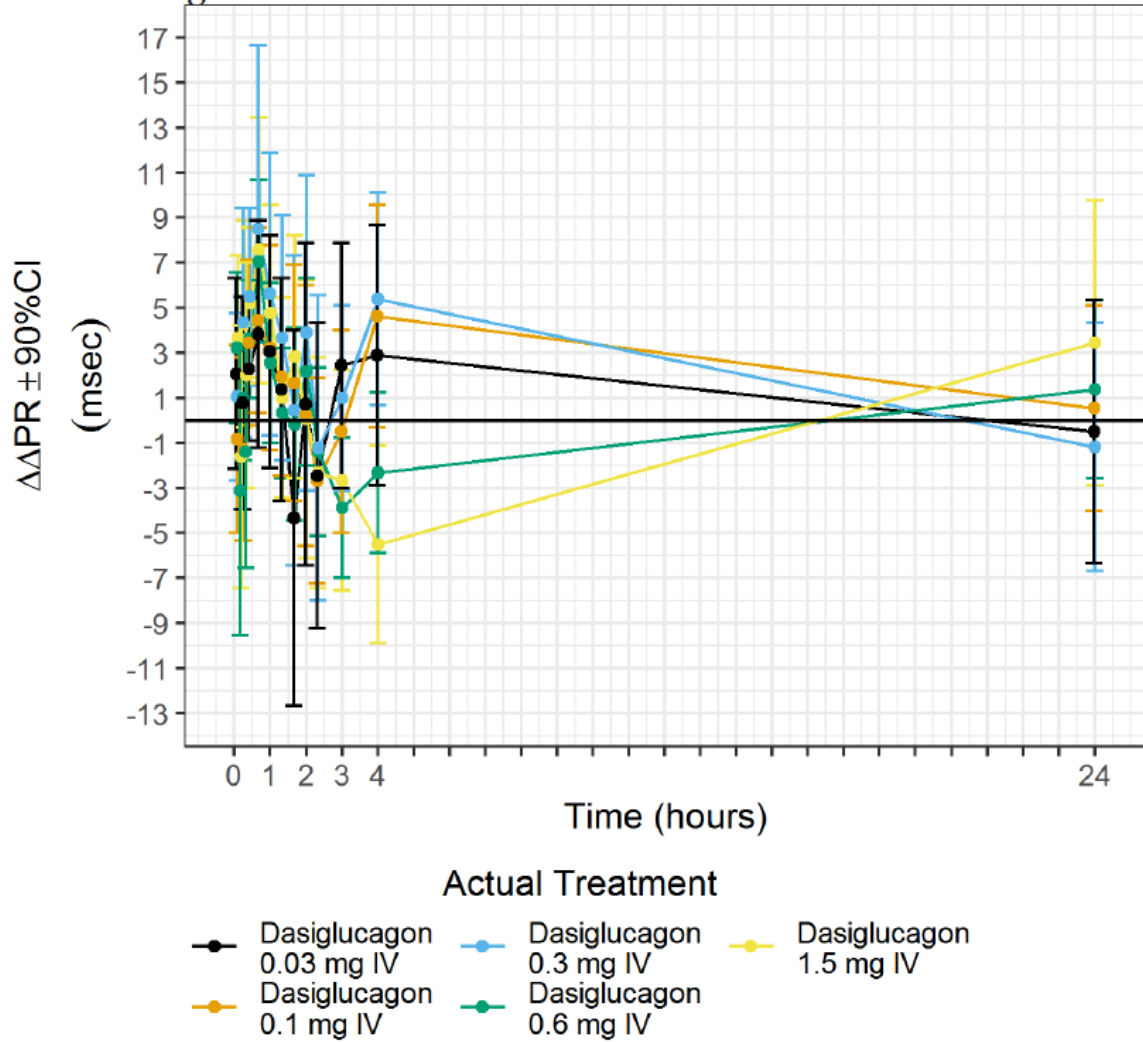
DCN Response: We do not believe that clinically meaningful PR interval effects are demonstrated. At supratherapeutic exposures (1.5 mg administered as a single intravenous infusion over 5 min, which covers two-times the worst-case exposure scenario), TQT study ZP4207-17144 demonstrated no clinically meaningful changes in the PR interval. No subject had a PR interval exceeding 220 msec. Per our review of sequential ECG tracings from subjects experiencing hemodynamic adverse events on the same day that an ECG abnormality was documented across the development program in dasiglucagon-treated subjects, there were no abnormal finding for PR interval effects. The ECG abnormalities that were noted involved transient non-specific ST-T-wave changes or minor variants from baseline. We agree with the sponsor's assessment that in 3 of the 4 cases where 12 lead ECGs were available (tracings not available for one of the four subjects), the PR interval "prolongation" was the output of an automated ECG mis-read.

2. We understand that T-wave inversion can be nonspecific; however, given the timing of the event post-dosing relative to the Cmax of dasiglucagon, we request your comment on the event as described above and whether it is clinically meaningful or warrants labeling.

DCN Response: Asymptomatic transient non-specific ST-T-wave changes can happen for a variety of reasons, including hyperventilation. Its occurrence in a single subject without clinical consequences is not a basis for a warning/precaution in labeling.

DATA FROM QT-IRT REVIEW OF TQT STUDY ZP4207-17144:

Figure 3: Median and 90% CI of $\Delta\Delta PR$ Timecourse



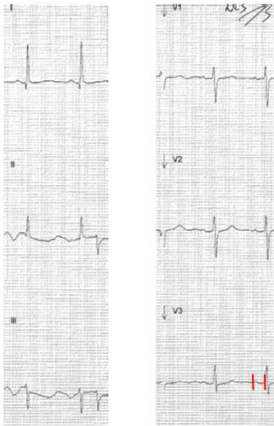
- No subject had PR > 220 msec

SPONSOR REANALYSIS OF FOUR SUBJECTS WITH PR INTERVAL OUTLIERS (from midcycle sponsor slides):

2. ZP4207- (b) (6) (0.6 mg dasiglucagon)

Dosing Day 1, 35 min post-dose ECG

Pre-dose PR: 168 ms



Investigator overall interpretation: abnormal, not clinically significant

Computer interpretation:
Ectopic atrial rhythm, PQ (PR) interval 236 msec

Zealand assessment:
Artifacts in in the limb leads (e.g. II and III) probably caused by incorrect attachment of the limb electrodes
Manual reading in lead V3:
Sinus rhythm, PR interval ~ 160 msec

Warning: artifact in (part of) recording - use interpretation with caution
ectopic atrial rhythm

page 1



Time of record: (b) (6) 12:33

2. ZP4207- (b) (6) (dasiglucagon)

Dosing Day 2, 20 min post-dose ECG

Pre-dose PR: 168 ms



Investigator overall interpretation: normal

Computer interpretation:
A-fib, PQ (PR) interval 382 ms

Zealand assessment:
Noise and baseline shift in the limb leads
Manual reading (lead V5):
Sinus rhythm, PR interval ~170 ms

Interval
RR 783 ms
P 382 ms
PQ
Time of record: (b) (6) 10:00

Interpretation
VORHOFFLATTERN
LAGETYP NORMAL

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2. ZP4207 (b) (6) (0.6 mg dasiglucagon)

Dosing Day 3, 45 min post-dose ECG

Pre-dose PR: 178 ms



Investigator overall interpretation: normal

Computer interpretation:
A-fib, PQ (PR) interval 372 msec

Zealand assessment:
Noise in the limb leads
Manual reading in lead V5:
Sinus rhythm, PR interval ~ 180 msec

Interv. / RR 1092 ms
P -
PQ 372 ms
Time of record: (b) (6) 10:15

Interpretation
VORHOFLIMMERN
LAGETYP NORMAL

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2. ZP4207 (b) (6) (1 mg GlucaGen)

Dosing Day 3, 35 min post-dose ECG

Pre-dose PR: 162 ms



Investigator overall interpretation: normal

Computer interpretation:
A-fib, PQ (PR) interval 372 msec

Zealand assessment:
Noise in the the limb leads
Manual reading (lead V5):
Sinus rhythm, PR interval ~ 175 msec

Interv. / RR 980 ms
P -
PQ 372 ms
Time of record: (b) (6) 10:40

Interpretation
VORHOFLIMMERN
LAGETYP NORMAL

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Reviewer's note: DCN agrees with the sponsor's interpretation that the outlier PR intervals for these subjects were automated mis-reads – they are not abnormal. The 12-lead ECG from subject ZP4207 (b) (6) (0.6 mg dasiglucagon) was not available for review.

ECG REVIEW (SPONSOR'S RESPONSE TO 14 SEP 2020 IR):

From program-wide data integration, four dasiglucagon-treated subjects experienced an abnormal ECG on the same day as a hemodynamic adverse event. None of these four cases involved symptoms referable to AV-nodal conduction failure. These cases are summarized as follows from the sponsor's description, followed by DCN ECG over-read analysis:

- Subject ID ZP4207-(b) (6). This 33 year-old subject experienced an adverse event of a vagal episode (described as 'presyncope' 77 min post-dose that was associated with bradycardia, nausea, sweating and paleness). ECG abnormalities included sinus arrhythmia at screening and sinus tachycardia 300 min post-dose.

Reviewer's comment: Review of this subject's ECG sequence shows no significant/acute changes, and no important PR interval prolongation.

- Subject ID ZP4207-(b) (6). This 36 year-old subject experienced an adverse event of blood pressure reduction from 122/80 mmHg pre-dose to 100/68 mmHg 88 minutes post-dose. ECG abnormalities at 20 and 60 minutes post-dose included borderline low R-wave amplitude.

Reviewer's comment: Review of this subject's ECG sequence shows no significant/acute changes, and no important PR interval prolongation.

- Subject ID ZP4207-(b) (6). The 24 year-old subject experienced an adverse event of palpitation 90 minutes post-dose. ECGs for this subject showed sinus arrhythmia 30 min post-dose and had a normal ECG at 360 minutes post-dose. No ECGs between 30 and 360 minutes post-dose were not acquired.

Reviewer's comment: Review of this subject's ECG sequence shows no significant/acute changes, and no important PR interval prolongation. However, no ECGs were acquired during the period that the subject was experiencing palpitation.

- Subject ID ZP4207-(b) (6). This 45 year-old subject experienced an adverse event of bradycardia day 1 and an adverse event of hypotension on dosing day 5. Predose ECGs were reported as normal at the pre-dose assessments on all five dosing days. Abnormal ECG findings were reported on most of the post-dose assessments – all were T-wave abnormalities, none involving AV-nodal conduction delay

Reviewer's comment: review of this subjects tracings demonstrated non-specific ST-T-wave changes only.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

PRESTON M DUNNMON
10/29/2020 03:05:25 PM

NORMAN L STOCKBRIDGE
10/29/2020 03:10:12 PM



Memorandum

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH
DIVISION OF CARDIOLOGY AND NEPHROLOGY

Date: July 28, 2020

From: Interdisciplinary Review Team for Cardiac Safety Studies

Through: Christine Garnett, Pharm.D.
Clinical Analyst, DCN

To: Elizabeth Godwin, RPM
DLO

Subject: IRT Consult to NDA-214231 (SDN001)

Note: Any text in the review with a light background should be inferred as copied from the sponsor's document.

This memo responds to your consult to us dated 4/13/2020 regarding the proposed labeling language in Section 12.2 of the label. We reviewed the following materials:

- Previous IRT review for IND-127866 dated 12/14/2017 in DARRTS ([link](#));
- Previous IRT review for IND-127866 dated 03/01/2019 in DARRTS ([link](#));
- Previous IRT review for IND-127866 dated 02/13/2020 in DARRTS ([link](#));
- Sponsor's clinical study report # ZP4207-17086 (SN0001 / SDN001; [link](#)); and
- Sponsor's proposed product label (SN0001 / SDN001; [link](#));

1 IRT Responses

Previously, the IRT reviewed sponsor's request for substitution of thorough QT study (Study # ZP4207-17144) and no significant QTc prolongation effect of dasiglucagon was detected in our previous QT assessment. The data is adequate to serve as a substitute for a thorough QT study with the proposed therapeutic dose of 0.6 mg.

2 Proposed Label

Below are proposed edits to the label submitted to SDN001 ([link](#)) from the IRT. Our changes are highlighted ([addition](#), [deletion](#)). Please note that this is a suggestion only and that we defer final labeling decisions to the Division.

12.2 Pharmacodynamics

Cardiac Electrophysiology

(b) (4) At the dose resulting in (b) (4) the concentration (b) (4) the recommended therapeutic dose, (b) (4) <TRADENAME> does not prolong the QT interval to any clinically relevant extent.

We propose to use labeling language for this product consistent with the “Clinical Pharmacology Section of Labeling for Human Prescription Drug and Biological Products – Content and Format” guidance.

The exposures achieved with the 1.5 mg (administered as a single intravenous infusion over 5 min) were higher than the exposures associated with 1 mg subcutaneous injection. In absence of dose proportionality data for subcutaneous route at higher exposures, bioavailability-corrected concentration-based exposure margin is proposed for the label.

3 Background

Zealand Pharma is developing dasiglucagon for the treatment of severe hypoglycemia in patients with diabetes (age ≥ 6 years; as a rescue treatment of severe hypoglycemia). Dasiglucagon (ZP4207, average mass: 3381.7 g/mol) is a peptide analog of human glucagon (with 7 amino acid substitutions compared to glucagon to enhance stability). The product is formulated as solutions (1 mg/mL; 0.6 mL) for injection using single-use pre-filled safety syringe or HypoPal (single-dose use). The proposed therapeutic dose is 0.6 mg (as single subcutaneous dose; age ≥ 6 years). The mean peak concentrations of 1510 pmol/L (in adults, ~ 5 ng/mL) and 1160 pmol/L (pediatric, ~ 4 ng/mL) are expected with the single 0.6 mg subcutaneous injection of dasiglucagon (Study # 17086).

Previously, the IRT reviewed sponsor’s request for substitution of thorough QT study (02/13/2020). The effect of dasiglucagon was evaluated in Study # ZP4207-17144 and no significant QTc prolongation effect of dasiglucagon was detected in the QT assessment. This was a randomized, double-blind, placebo-controlled, dose-escalation study in healthy subjects. The highest dose evaluated was 1.5 mg administered as a single intravenous infusion (over 5 min), which covers two-times the worst-case exposure scenario (section 3.1 of the previous review) and therefore supported waiving the requirement for inclusion of a positive control.

The data were analyzed using exposure-response analysis as the primary analysis, which did not suggest that dasiglucagon is associated with significant QTc prolonging effect (refer to section 4.5 of the previous review) – see Table 1 for overall results.

Table 1: The Point Estimates and the 90% CIs (FDA Analysis)

ECG Parameter	Treatment	Concentration (pmol/L)	$\Delta\Delta\text{QTcF}$ (msec)	90% CI (msec)
QTc	0.6 mg (SC [#])	1534.0	0.7	(-1.1 to 2.5)
QTc	1.5 mg (IV [*])	57895.6	-0.1	(-4.2 to 3.9)

**administered as a single intravenous dose of dasiglucagon using 5 min infusion; #administered as a single subcutaneous dose of dasiglucagon; For further details on the FDA analysis please see section 4 of our review.*

The findings of this analysis were further supported by the available nonclinical data (section 3.1.2 of the previous review) and categorical analysis (section 4.4 of the previous review). In addition,

our independent analysis did not suggest the presence of significant negative treatment bias (section 4.2.2 of the previous review). The peak concentrations observed in this study following intravenous administration of 1.5 mg dose offers >2-fold margin over the worst-case scenario in patient population with subcutaneous administration.

Recently, the sponsor conducted a phase 3, randomized, double-blind, placebo- and active-controlled, parallel-arm study evaluating the efficacy, safety, and pharmacokinetics of dasiglucagon in pediatric population (Study # ZP4207-17086). The dasiglucagon plasma concentrations were measured on Day 1 following single 0.6 mg injection for treating hypoglycemia in children with type 1 diabetes mellitus. The peak concentration of 1330 ± 724 pmol/L (n=20; Tmax: ~0.5 h, half-life: 0.7 h) were observed in pediatric population. Slightly higher peak concentrations were observed in lower age group (n=8; 1644 pmol in 6 to 11 years) compared to that observed with the higher age group (n=12; 919 pmol in 12 to 17 years). However, these concentrations levels are covered in the previous assessment.

Following subcutaneous doses, dasiglucagon exhibits a dose proportional pharmacokinetics between doses 0.1 and 1 mg (Study # 15126). However, the exposures achieved with the 1.5 mg (administered as a single intravenous infusion over 5 min; Cmax: 57895.6 pmol/L) were higher than the exposures associated with 1 mg subcutaneous injection. In absence of dose proportionality data for the subcutaneous route at higher doses, bioavailability-corrected concentration-based exposure margin is proposed for the label. The bioavailability of dasiglucagon following subcutaneous administration (0.6 mg in 0.6 mL) is approximately $\frac{(b)}{(4)}$ % (Study # ZP4207-17144).

Thank you for requesting our input into the development of this product. We welcome more discussion with you now and in the future. Please feel free to contact us via email at cdcrpqt@fda.hhs.gov.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

GIRISH K BENDE
07/28/2020 03:22:21 PM

CHRISTINE E GARNETT
07/28/2020 03:23:43 PM