EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

EMA/CHMP/50784/2022
Human Medicines Division

Type IB variation report

COMIRNATY	EMEA/H/C/005735/IB/0106/G
INN / Common name	tozinameran
Scope as per guideline	B.I.b.1.c - Change in the specification parameters and/or limits of an AS, starting material/intermediate/reagent - Addition of a new specification parameter to the specification with its corresponding test method B.I.b.2.c - Change in test procedure for AS or starting material/reagent/intermediate - Other changes to a test procedure for a reagent, which does not have a significant effect on the overall quality of the AS
Precise scope	B.I.b.1.c (IB) - To add Activity test method acceptance criteria to the specifications of active substance raw materials listed below, used in the manufacturing process of the active substance Tozinameran at Pfizer, Andover. The limits have been set as follows: - DNase I: [redacted] - Proteinase K: [redacted] - Pyrophosphatase [redacted] - RNase inhibitor: [redacted] - T7 polymerase [redacted] B.I.b.2.c (IB) - To add the in-house Purity test procedure for the DNase I, Pyrophosphatase, RNase inhibitor and T7 polymerase raw materials used in the manufacturing process of the active substance Tozinameran, at Pfizer, Andover. In addition, the MAH included small-scale study data, REC 7. REC 3: "The MAH should implement in-house functional activity analytical methods for release testing of enzymes used in the manufacturing process at all relevant manufacturing sites" is partially fulfilled.

	REC 7: "The MAH should provide the results of the studies performed to enhance the robustness of the DNase digestion step" is partially fulfilled.
Annexes affected	None
EU numbers affected	EU/1/20/1528/001-005
Rapporteur	Filip Josephson
Procedure manager	[redacted]
Contact person (if LoA provided)	n/a
eCTD sequence related to the procedure	0284, 0321

Status of the procedure		
	Application received by the EMA on	24 January 2022
	Start of the procedure	25 January 2022
☒	This report is sent to the Rapporteur for assessment by	13 February 2022
☒	Request for Supplementary Information (RfSI) (see section 2.2)	
	Responses to RfSI were received on	9 March 2022
☒	This report is sent to the Rapporteur for assessment by	29 March 2022
☒	Final updated AR	31 March 2022

1. Validation

1.1. Checklist

		Yes	No	N/A
APPLICATION FORM	**Present dated and signed by the authorised contact person (or letter of authorisation is provided).**	x		
	States the correct name and address of the MA Holder and of the contact person.	x		
	EU numbers of all affected presentations are correctly listed in the Application Form, Annex A and Product Information.	x		
	All changes applied for are correctly classified.	x		
	Identical classification scopes are indicated as many times as needed (e.g. new pack size, new sites).			x
	Relevant conditions to be fulfilled and documentation are ticked (if applicable).	x		
	'Precise scope' includes detailed description of change(s). In case of grouping also includes corresponding classification scopes.	x		
	'Present/Proposed table' (or attachment) reflects all changes	x		

		Yes	No	N/A
	applied for, dossier section numbers refer to the lowest possible level and include the precise current and proposed wording as in the relevant sections of the dossier and, if applicable, in the Product Information and/or RMP.			
	Annexes affected are correctly selected.			x
	Declaration of the Applicant: Boxes 1 and 2 **are ticked and date of implementation is stated.**	x		
GROUPING	**Grouping is acceptable, as outlined in Annex III of Reg. (EC) No 1234/2008 or agreed with the Agency.**	x		
DOCUMENTATION	*Only the relevant documents are included.* [1a, b]	x		
ASMF [2]	**ASMF Holder has submitted the applicant's and/or restricted part.**			x
	EMA or EU ASMF number is included in the 'Present/Proposed' table.			x
New indications of a generic medicinal product	**For new indications falling under an orphan designation, similarity report (and derogation claim, if applicable) is included.**			x

[1a] For variations implementing PI text agreed with Competent Authority: copy of the request or previous assessment is included as attachment to the cover letter or application form.

[1b] For variations implementing PI text of a new indication of an originator product, and if there are orphan authorised medicinal products for a condition related to the proposed new indication, similarity report is included in Module 1.7.1.

[2] See EMA pre-submission guidance Q24 for further information on ASMF submission and EMA/EU ASMF number.

[3] The final product information i.e. Annex I, II, IIIA, IIIB and A, must be submitted electronically as one clean PDF file for each EEA language (see also the User guide on the preparation of PDF versions of the product information). The Annexes should be presented in strict compliance with the QRD Convention.

[4] Mandatory in case of update to the latest RMP template.

Note: For new indication for a generic, please check that the indication of the reference medicinal product is not under data protection (e.g. see Art. 10(5), Art 74. of Dir 2001/83/EC).

Note on text: **Bold**: blocking validation issue; Italics: information needed for documentation check but not blocking; Normal: Information considered for completeness of submission, not blocking but MAH may be reminded in variation report to address for future submissions.

Issues (related to the checklist) raised during validation:

Classification	Issues identified	Resolved/Comments/For info
B.I.b.1.c	-	
B.I.b.2.c	-	Variation B.I.b.2.c to implement the purity in-house test procedures for DNase I, Pyrophosphatase, RNase inhibitor and T7 polymerase: No description of the test methods is included in 3.2.S.2.3. This is not commented upon during validation but can be further addressed during assessment, as needed.

Classification	Issues identified	Resolved/Comments/For info
REC 7	-	As it was recommended that REC3 and REC 7 are grouped in future submission, the results of the studies performed to enhance the robustness of the DNase digestion step are accepted as part of this grouping.

1.2. Validation outcome

☒ Satisfactory

☐ Additional information to be provided before the start of the procedure (see above sections 1.1 and 1.2)

☐ Satisfactory after receipt of the validation responses

☐ Unsatisfactory, issues not addressed in the responses received, based on the following grounds:

2. Assessment

2.1. Initial submission

The Sponsor has submitted an application for a grouped type IB variation to fulfil REC#3. Along with this submission a PAM-REC is being submitted (see 1.0 PAM-Form for REC07) to fulfil the Recommendation 7 (REC7) as listed in the EPAR.

This grouping of variations proposes acceptance criteria for activity testing of the enzymes that are utilized as raw materials in the drug substance manufacturing process (DNase I, Proteinase K, Pyrophosphatase, RNase inhibitor and T7 polymerase). Additionally, the analytical procedure used for purity test is updated for all enzymes except Proteinase K.

All the information provided with this submission both for fulfilment of REC7 and REC3 concerns Pfizer, Andover. It is anticipated that the information for fulfilment of REC3 for the other drug substance manufacturing sites (ie, BNT Marburg and BNT Mainz-Rentschler) will be provided in Q2 2022.

2.2. Updates of the dossier

Section 3.2.S.2.3 control of Materials- Materials Used in Manufacture (Andover) has been updated with the test method acceptance criteria to enzymes used as raw materials. In addition, Module 1 has been updated with Responses to fulfil REC 3 and REC 7.

2.3. Control of Materials (3.2.S.2.3) - Materials Used in Manufacture (Andover)

This variation introduces numerical activity values for each of the drug substance enzymes as per Rec #3. The acceptance criteria have been updated from "report results" to a numerical value for each enzyme based on the number of distinctly different enzyme batches received for each enzyme. Analytical procedures, for activity testing of the enzymes (DNase I, Proteinase K, Pyrophosphatase, T7 polymerase)

and RNase inhibitor that are utilized as raw materials in the drug substance manufacturing process, have been developed, validated and implemented.

Table 3.2.S.2.3-3. Acceptance Criteria for Non-Compendial Starting Materials and Raw Materials Used in Manufacturing

Material	Characteristic	Acceptance Criteria
5'-cap solution [a]	Identity [b]	Identity confirmed
	Purity	[redacted]
	Concentration	
Ammonium sulfate	Identity[b]	Meets Requirements
ATP solution[a]	Identity[b]	Identity confirmed
	Purity	[redacted]
	Concentration	
CTP solution[a]	Identity[b]	Identity confirmed
	Purity	[redacted]
	Concentration	
DL-Dithiothreitol	Identity[b]	Meets Requirements; Spectrum exhibits maxima at same wavelengths as that of reference
	Appearance (color)	White
	Appearance (form)	Powder
	Purity	[redacted]
DNase I[c]	Identity[b]	Identity confirmed
	Activity[b]	[redacted]
	Purity [b]	
GTP solution[a]	Identity[b]	Identity confirmed
	Purity	[redacted]
	Concentration	
HEPES	Appearance[b]	White crystals or crystalline powder (Passes test)
	Identity[b]	Passes test
HEPES sodium salt	Appearance[b]	White powder
	Identity[b]	Spectrum is consistent with reference spectrum
Magnesium acetate tetrahydrate	Identity[b]	Meets Requirements

Material	Characteristic	Acceptance Criteria
	Appearance (color)	White
	Appearance (form)	Powder to crystalline powder
N1-methylpseudo UTP solution[a]	Identity[b]	Identity confirmed
	Purity	[redacted]
	Concentration	
Proteinase K[c]	Identity[b]	Identity confirmed
	Activity[b]	[redacted]
Pyrophosphatase[c]	Identity[b]	Identity confirmed
	Activity[b]	[redacted]
	Purity[b]	
RNase inhibitor[c]	Identity[b]	Identity confirmed
	Activity[b]	[redacted]
	Purity [b]	
Spermidine	Identity[b]	Identity confirmed
	Appearance (color)	White/colorless to light yellow
	Appearance (form)	Clear liquid/solid
	Purity	[redacted]
T7 polymerase[c]	Identity [b]	Identity confirmed
	Activity[b]	[redacted]
	Purity [b]	

a. Starting material
b. Test is performed by or on behalf of the drug substance manufacturer
c. 50% glycerol in buffer solution

This testing is performed on behalf of the drug substance manufacturer (Pfizer, Andover) to confirm that activity of each enzyme is within an acceptable range. A brief description of these analytical procedures is provided in Module 1 REC 3 and REC 7 Responses. These analytical activity assays, performed by the contract laboratory have a different activity unit definition than what is utilized by and reported on the vendor Certificate Of Analysis (COA) for each enzyme.

The acceptance criteria have been updated from "report results" to a numerical value for each enzyme based on a small number (less than 10) of distinctly different enzyme batches received for each enzyme, with the exception of DNase I. Once testing results derived from 10 different batches of each of the enzymes (Pyrophosphatase, T7 polymerase, and Proteinase K) and RNAse inhibitor are available, the MAH will re-evaluate the acceptance criteria using statistical analyses coupled with manufacturing knowledge.

[redacted]

2.4. Post Authorisation Measure – CHMP REC #3 (Module 1)

Both the variations proposed to define the enzymes acceptance limits for activity, and the response below pertains to enzymes used in the drug substance (DS) manufacturing process at the Pfizer, Andover site. Pfizer has worked with a contract laboratory to develop methods and establish acceptance criteria for release of enzymes used in drug substance production.

These same test methods will also be transferred to a contract laboratory in Europe for testing enzymes used in the BioNTech Marburg and Mainz DS manufacturing process to confirm proposed acceptance limits. Updates for these sites will be provided in a subsequent submission.

Table 1. Method Description Comparison

Enzyme	Thermo Activity Method Short Description	Implemented Counter Testing Short Description
T7 Polymerase	T7 RNA polymerase is the enzyme that catalyzes RNA transcription using plasmid template and NTP to produce single stranded RNA molecule. One unit of T7 RNA polymerase is defined by the amount of enzyme which catalyzes 1 nmol of AMP incorporation into polynucleotide in 60 min. at 37°C. Activity is measured under these conditions: 40 mM Tris-HCl (pH 8.0), 6 mM MgCl2, 10 mM DTT, 2 mM spermidine, 0.5 mM of each NTP, 0.6 MBq/ml [3H]-ATP, 20 µg/ml plasmid DNA with RNA polymerase promoter. Transcript with incorporated [3H]- ATP is bound to positively charged nylon membrane and radioactivity is measured using liquid scintillation counter.	This procedure is based on the measurement of (pyrophosphate) PPi levels that are generated in a coupled reaction with in vitro T7 RNA transcription. In this assay, pSPT18-neo-DNA, which has been linearized with Pvu II, is used as the DNA template. The DNA template is subjected to in vitro transcription with a nucleotide mix containing ATP, CTP, GTP and UTP under standard conditions. The PPi that is generated is then measured using a pyrophosphate assay based on a proprietary pyrophosphate sensor that has its absorbance intensity proportionally dependent upon the concentration of pyrophosphate.
Pyrophosphatase	Pyrophosphatase (or inorganic pyrophosphatase) is an enzyme that catalyzes the conversion of one molecule of pyrophosphate to two phosphate ions. Activity assay of Inorganic Pyrophosphatase is performed by observing a unique blue-colored complex which is formed of phosphate with molybdate and reducing agents. A deep blue color is developed due to the unreduced phosphomolybdate complex and its absorbance is measured at 820 nm. The concentration (nmol) of phosphate is estimated using a phosphate standard curve.	The activity of Pyrophosphatase is determined by spectroscopy, with absorbance measured at 660 nm. Inorganic Pyrophosphatase catalyzes the hydrolysis of inorganic pyrophosphate into two orthophosphates. Pyrophosphates (PPi) are produced by a number of biochemical reactions, such as ATP hydrolysis, DNA and RNA polymerization, cyclic AMP formation by the enzyme adenylate cyclase, and the enzymatic activation of fatty acids to form their coenzyme A esters. The concentration (µmol) of phosphate is estimated using a standard curve.
DNAse I	DNase I, RNase-free, AOF, is an endonuclease that digests single- and double-stranded DNA. It hydrolyzes phosphodiester bonds producing mono- and oligodeoxyribonucleotides with 5'-phosphate and 3'-OH groups. Concentration assay of recombinant DNase I is performed by observing DNA substrate concentration during 2 minute span. DNA concentration decreases as DNase I degrades DNA and its absorbance is measured at 260 nm. The concentration (U/µl) of DNase I is estimated using a DNase I standard. Definition of Activity Unit: One unit of the enzyme completely degrades 1 µg of plasmid DNA in 10 min at 37 °C.	DNase I is an enzyme that hydrolyzes the bonds between nucleotides in DNA. Hydrolysis of these bonds separates the DNA double helix into two separate DNA strands. Nucleic acids strongly absorb light at 260 nm because of the structure of purine and pyrimidine bases increasing the absorbance at 260 nm. This assay will convert the change in Absorbance at 260 nm into activity units of DNase I using DNA from Bovine Calf Thymus.

Enzyme	Thermo Activity Method Short Description	Implemented Counter Testing Short Description
RNAse Inhibitor	RNase Inhibitor, AOF, is a protein that specifically binds to the RNase enzyme, resulting in inhibition of RNase catalytic activity (ability to hydrolyse RNA). Activity concentration for RNase Inhibitor, AOF, is determined as an ability to inhibit a specified amount of RNase A. After incubation, [3H] labeled RNA substrate is precipitated with trichloracetic acid. Degraded [3H] RNA, which stays in solution, is determined by radioactivity measurement. Regression analysis with [redacted] is performed to determine activity concentration value of RNase Inhibitor, AOF. The equation used in the regression analysis is provided below. where: y — radioactivity x — RNase Inhibitor activity concentration d — asymptote of the sygmoid function, which corresponds to radioactivity when RNase A is without any inhibition c — asymptote of the sygmoid function, which corresponds to radioactivity when RNase A is fully inhibited a – Hill coefficient b – inflection point, corresponding to half of the substrate degradation compared to full inhibition. This parameter is	RNase Inhibitor is a protein that binds to RNase enzymes and inhibits their ability to denature RNA. The inhibitory activity of RNase Inhibitor (RiboLock) is determined using a commercially available RNase detection kit with a fluorometric RNA substrate. The measure of inhibition is determined by comparing a solution prepared at a level that is theoretically fully inhibited to that of an uninhibited RNase solution. The result is calculated as percent of inhibited RNase A.
Proteinase K	(This information provided by [redacted] who releases the material to Thermo). The specific activity of Proteinase K is ≥ U/mg, when assayed with Chromozym assay (equivalent to ≥ 30 U/mg with the hemoglobin assay).	Proteinase K releases amino acids and peptides when incubated with a hemoglobin substrate at 37°C. The released amino acids and peptides are equivalent to 1 µmol of tyrosine for 1 minute of these assay conditions. This can be colorimetrically detected with Folin & Ciocalteu's Phenol Reagent at 750 nm.

AMP = adenine monophosphate; AOF = Animal origin free; ATP = adenine triphosphate; DNA = deoxyribonucleotide acid; CTP = cytosine triphosphate; GTP = guanosine triphosphate; NTP = nucleotide triphosphate; UTP = uridine triphosphate

For DNase I: There have been a minimum of 10 distinctly different batches of DNase I received from the current vendor (ThermoFisher) and Pfizer, Andover is establishing the acceptance criteria using this data generated from the counter testing method that is in use for routine release testing of the raw material enzymes. A [redacted] standard deviation (SD) from the mean value of the data set being evaluated, which provides a confidence in coverage in the population, has been used to establish the acceptance criteria for DNase I, using these 10 different batches of DNase I enzyme, along with a subset of results generated on sub batches of some of the parent batches. Additionally, the activity counter testing method, established and run by the contract laboratory, Eurofins Lancaster Laboratories Inc, on behalf of Pfizer, Andover is currently undergoing assessment and revision for optimization. While no previous release results generated will be retested/re-reported as a part of the method optimization activities, future release results may have lower activity values than previously seen. Therefore, the subset of data used to establish the [redacted] acceptance criteria for DNase I includes both the original value from release testing as well as what the re-calculated value would be from the optimized method. [redacted]

For T7 polymerase, pyrophosphatase, Proteinase K enzymes and RNase Inhibitor, the Applicant has not yet received 10 distinctly different batches of each material from the current vendor, ThermoFisher. The acceptance criteria for activity testing of each of these materials were set based on the minimum and the maximum values of activity observed for the material when used in the manufacturing process that led to

the generation of the drug substance, which met all the release acceptance criteria.

The following acceptance criteria are being proposed:

- T7 Polymerase:
- Pyrophosphatase:
- RNase Inhibitor:
- Proteinase K:

If future in-house testing results in an activity value outside of the above listed acceptance criteria, then an evaluation on a small-scale drug substance will be completed to support use. If the small scale drug substance meets the release acceptance criteria, then the enzyme batch would be deemed acceptable for use in at scale manufacturing and regulatory actions will be taken accordingly.

When a minimum of 10 distinctly different batches of the above enzymes (T7 polymerase, pyrophosphatase, and Proteinase K) and RNase inhibitor have been received and tested, the Applicant will re-evaluate the acceptance criteria based upon statistical analyses coupled with manufacturing knowledge. Additionally, if the Applicant establishes secondary suppliers for the enzymes or RNase inhibitor for use in the manufacturing process, the Applicant commits to submitting the variation(s) with described changes, including any proposed changes to acceptance criteria that may be warranted. The variation(s) will include any updated information on acceptance criteria that may be available at the time of filing.

[redacted]

No changes to the DNAse I digestion step were recommended at the time based on the results from these studies.

New studies at small scale have been initiated and are focused on combining multiple parameter changes to increase the DNAse I digestion step robustness to account for raw material variability. [redacted]

To that end, minor adjustments, within the filed parameters, to both the IVT and DNase I digestion steps have been implemented at all drug substance manufacturing sites based on scientific rationale to improve DNase I digestion performance. Results from these drug substance manufacturing scale adjustments show consistent residual DNA levels.

As additional data about raw material variability and impact from process parameters is gathered, adjustments to the process outside of currently filed ranges would be properly communicated and filed.

Manufacturing experience and small-scale data show that the residual DNA levels are impacted by [redacted] Furthermore, while Pfizer has had the

opportunity to test 10 lots of DNase I activity with the additional activity assay, only two lots and a total of 4 shipments have gone into the manufacturing process. Based on the limited DNase I lots used at small and at-scale commercial DS manufacturing process data, it is difficult to make a correlation to residual DNA.

The current control strategy enhancements include independent activity testing with defined acceptance criteria for DNase I and other enzymes, and minor adjustments to the DS manufacturing process, ensuring DS manufacturing process is consistent in delivering acceptable process performance, including robust DNA removal.

Assessment:

Table 3.2.S.2.3-3 in Section 3.2.S.2.3 has been updated to define acceptance criteria for activities of the enzymes used in the DS manufacturing process. Additional information on the methods to determine enzyme activity and how the acceptance criteria were set are provided in the response document in Module 1. In the assessment of EMEA/H/C/005735/IB/0055 it was stated that to complete REC #3, the Applicant should provide short descriptions of the methods applied and present data to demonstrate adequate correlation between the activity results reported by the vendor and the implemented methods. No correlation between results reported by the vendor and those obtained by the implemented methods are provided. However, since the MAH commits to perform the tests, using established acceptance criteria, the information is considered acceptable. [redacted]

- However, the method descriptions should be included in section 3.2.S.2.3 of the dossier. **(OC)**

Regarding the defined acceptance criteria, for DNase I there have been 10 different batches applied in the process and the MAH have used mean value [redacted] to define acceptable ranges. For T7 polymerase, pyrophosphatase, RNase Inhibitor and Proteinase K, preliminary acceptance criteria have been proposed, that might be revised after a sufficient number of batches have been included in the manufacturing process. This is found acceptable.

All the information provided with this submission for fulfilment of REC#3 concerns Pfizer, Andover. The MAH anticipates that information for fulfilment of REC#3 for the other drug substance manufacturing sites (ie, BNT Marburg and BNT Mainz-Rentschler) will be provided in Q2 2022. Thus, REC#3 can only be considered as acceptable once this information has been provided and a new date for this submission should be set.

- REC#3 can only be considered as acceptable once information for the two European drug substance manufacturing sites (ie, BNT Marburg and BNT Mainz-Rentschler) has been provided. The MAH should suggest a date for this submission. **(OC)**

Regarding REC #7, a summary of the small-scale analyses is provided, and implementation decision is explained for all investigated process parameters. The MAH also explains that new small-scale studies have been initiated to increase the DNAse I robustness. It is also explained that manufacturing experience and small scale data show that the residual DNA levels are impacted by [redacted] However, since small-scale studies are ongoing, results from these are requested in order to fulfil REC#7.

- The MAH states that new small-scale studies have been initiated to increase the DNAse I robustness. In order to fulfil REC#7, data and conclusions from these studies should be provided. [redacted] the correlation between DNAse I activity, residual DNA as measured by the in-house methods and possible other parameters should

be sufficienty evaluated and discussed. **(OC)**

Active substance master file (ASMF)

Not applicable.

Conclusion:

☐ The changes proposed by the MAH are accepted and the variation(s) is/are approvable.

☒ The changes proposed are not accepted and the MAH may amend the application within 30 days in order to address the issues outlined in Section "2.2. Request for Supplementary Information", before the variation(s) can be approved.

2.5. Request for supplementary information (RfSI)

List of Issues to be resolved:

1. Method descriptions of the methods to determine activities of the enzymes used the DS manufacturing process should be included in section 3.2.S.2.3 of the dossier.

2. REC#3 can only be considered as acceptable once information for the two European drug substance manufacturing sites (ie, BNT Marburg and BNT Mainz-Rentschler) has been provided. The MAH should suggest a date for this submission.

3. The MAH states that new small-scale studies have been initiated to increase the DNAse I robustness. In order to fulfil REC#7, data and conclusions from these studies should be provided [redacted] the correlation between DNAse I activity, residual DNA and possibly other parameters should be sufficienty evaluated and discussed.

2.6. Responses to RfSI

Question 1:

Method descriptions of the methods to determine activities of the enzymes used the DS manufacturing process should be included in section 3.2.S.2.3 of the dossier.

Summary of the MAH responses:

Section 3.2.S.2.3 Control of Materials- Materials Used in Manufacture [Andover] was updated to include information on the analytical procedures for the enzymes and the RNase inhibitor used in the drug substance manufacturing process. The section was also reformatted to improve clarity of the information presented.

Assessment of the responses:

The Applicant states that section 3.2.S.2.3 Control of Materials- Materials Used in Manufacture [Andover] was updated to include information on the analytical procedures for the enzymes and the RNase inhibitor. However, only the type of methods are tabulated, e.g. "spectroscopy". Short method descriptions in line with those provided in Table 1 in the response document from 24 January 2022 should be included in section 3.2.S.2.3 of the dossier. This update can be done in connection with the variation for fulfilment of REC #3, at the latest 30 Jun 2022, refer to Question 2.

Conclusion

Issue not solved, but not further pursued with this variation.

Short method descriptions in line with those provided in Table 1 in the response document from 24 January 2022 should be included in section 3.2.S.2.3 of the dossier. This update can be done in connection with the variation for fulfilment of REC #3, at the latest 30 Jun 2022, refer to Question 2.

Question 2:

REC#3 can only be considered as acceptable once information for the two European drug substance manufacturing sites (ie, BNT Marburg and BNT Mainz-Rentschler) has been provided. The MAH should suggest a date for this submission.

Summary of the MAH responses:

The variation for the fulfilment of REC #3 of the BioNTech sites Mainz/Rentschler and Marburg is target for submission by 30 Jun 2022.

Assessment of the responses:

The Applicant suggests to submit the variation for the fulfilment of REC #3 of the BioNTech sites Mainz/Rentschler and Marburg by 30 Jun 2022. This is found acceptable.

Conclusion

Issue solved, but REC #3 remains.

Question 3:

The MAH states that new small-scale studies have been initiated to increase the DNAse I robustness. In order to fulfil REC#7, data and conclusions from these studies should be provided. [redacted] the correlation between DNAse I activity, residual DNA and possibly other parameters should be sufficiently evaluated and discussed.

Summary of the MAH responses:

The MAH would like to clarify that previously submitted information to the Agency on 24 January 2022 was intended to provide an overview of completed small-scale studies.

The original DNase I digestion studies (2020) progressed significantly and assessed impact of [redacted] as summarized in Table 2 and Figure 1 (refer to EMEA/H/C/005735/IB/0106/G, sequence 0284) of the previous response. These studies were halted once a potential cause for the [redacted] residual DNA levels was identified. Additional small-scale studies were initiated when [redacted] residual DNA results were seen in manufacturing again.

The "new" studies further assessed, in a DOE format, the impact of a combination of process parameters on the DNase I digestion robustness. The results of these studies indicate that [redacted] as shown in EMEA/H/C/005735/IB/0106/G, sequence 0284 is an important contributor to residual DNA level.

Taken collectively, the results of small-scale studies and manufacturing experience indicated that residual DNA levels are affected by [redacted]

Based on the small-scale confirmation [redacted] n residual DNA levels and scientific rationale, minor adjustments, within the acceptance criteria as described in section 3.2.S.2.2 Manufacturing Process (Andover), [redacted] have been implemented

at all drug substance manufacturing sites to improve [redacted] Additionally, control strategy was enhanced by introducing independent activity testing with defined acceptance criteria for DNase I (and other enzymes). Results from the drug substance manufacturing scale adjustments show consistent residual DNA levels in drug substance. Data from drug substance batches (one manufacturing site) before and after the adjustments are shown in Figure 1.

[redacted]

The enhanced process controls implemented result in acceptable levels of residual DNA, ensuring manufacturing process consistency and quality of the drug substance. No new small-scale experiments are planned or considered to be needed to show correlation between DNase I activity with residual DNA.

If additional information on raw material variability and impact from process parameters require investigation, additional adjustments to the process operations and controls would be considered, communicated, and filed.

Assessment of the responses:

The Applicant explains that the main conclusion from the small-scale studies and manufacturing experience is that residual DNA levels are affected by [redacted]

Based on the small-scale confirmation [redacted] n residual DNA levels and scientific rationale, minor adjustments within the acceptance criteria as described in section 3.2.S.2.2 Manufacturing Process (Andover), [redacted] have been implemented at all drug substance manufacturing sites to improv[redacted] Data from drug substance batches (one manufacturing site) before and after the adjustments is provided in the response document. In order to complete REC #7, data from the two additional sites Mainz/Rentschler and Marburg should be provided. Section 3.2.S.2.5 of the dossier should be updated with data on residual DNA levels from all three sites. In addition, section 3.2.S.2.2 should be updated with information on the enhanced process control. If possible, the minor adjustments should be reflected in

the related acceptance criteria.

Conclusion

Issue not further pursued with this variation application, but REC #7 remains.

The Applicant should submit the data requested to solve REC #7 together with the variation for fulfilment of REC #3, at the latest 30 Jun 2022 (refer to Question 2).

In order to complete REC #7, data from the two additional sites Mainz/Rentschler and Marburg should be provided. Section 3.2.S.2.5 of the dossier should be updated with data on residual DNA levels from all three sites. In addition, section 3.2.S.2.2 should be updated with information on the enhanced process control. If possible, the minor adjustments should be reflected in the related acceptance criteria.

2.7. Specific Obligations and Recommendations

No specific obligation is related to this Type IB variation. The related recommendations are described below.

Recommendation	Status
Active substance	
3. The MAH should implement in-house functional activity analytical methods for release testing of enzymes used in the manufacturing process at all relevant manufacturing sites, by Q1 2021.	**Partly fulfilled** eCTD seq 0041: Request to extend the due date from Q1-2021 to Q2-2021. Agreed by email 16.03.2021. REC/027: note link to REC 7. It is recommended that REC3 and REC 7 are grouped in future submission. VAR IB-55: The methods cannot be regarded as fully implemented as long as the correlations to the activity results reported by the vendor have not been established. The Applicant should provide short descriptions of the methods applied and present data to demonstrate adequate correlation between the activity results reported by the vendor and the implemented methods. In addition, the specification limits should be defined by numerical values for all five activity tests. VAR IB-106-G: Information from the two European drug substance manufacturing sites (ie, BNT Marburg and BNT Mainz-Rentschler) should be provided by June 30 2022. Short descriptions of the methods to determine activities of the enzymes used the DS

Recommendation	Status
	manufacturing process should be included in the dossier (all sites).
7. The MAH should provide the results of the studies performed to enhance the robustness of the DNase digestion step in the active substance manufacturing process.	**Partly fulfilled** REC/027 ongoing, CHMP conclusion 20/05/2021: Further actions are required to fulfil Recommendation 7 including submission of a detailed summary of the results from the studies and inclusion of these data in Module 3.2.S.2.5 of the dossier by the end of second quarter 2021. It also recommended that Recommendations 3 and 7 are grouped. VAR IB-55: No results are provided, since the Applicant considers the small-scale study to be inconclusive and no adjustment to the DNase digestion step is recommended. This is not found acceptable, and data should be provided to support that no change is needed. In addition, the correlation of DNase I activity and levels of Residual DNA template as measured by the in-house methods should be sufficiently evaluated. VAR IB-106-G: In order to complete REC #7, data from the two additional sites Mainz/Rentschler and Marburg should be provided. Section 3.2.S.2.5 of the dossier should be updated with data on residual DNA levels from all three sites. In addition, section 3.2.S.2.2 should be updated with information on the enhanced process control. If possible, the minor adjustments should be reflected in the related acceptance criteria.

3. Overall conclusion

Based on the review of the data, the change(s) proposed by the MAH

☒ is/are approvable.

☐ is/are not approvable based on the following grounds:

Rapporteur's assessor:	Name: [redacted] Tel: [redacted] Email: [redacted] Date: 2022-03-29

☒ The assessor confirms that proprietary information on, or reference to, other products are not made in this assessment and that [redacted] on behalf of the Rapporteur: Filip Josephson endorses this report.