Case studies

Case study 1

Stability issues: "Catastrophin"

Presentation of the product

The Marketing Authorisation applicant (MAA) has submitted a dossier for a Marketing authorisation application for a biological medicinal product containing *"Catastrophin"* as active substance.

Catastrophin is a pegylated rec. growth factor manufactured from E.coli. It has one disulfide bridge and a MW of approx. 32.000 Da. Pegylation is used to prolong half-life and to enable weekly administration instead of daily.

The protein is extremely pH sensitive, in the characterization studies it was demonstrated that significant depegylation occurs below pH 7. Significant deamidation was observed above pH 7.0
The drug substance is defined as a bulk solution containing Catastrophin. It is filled in pre-sterilized polycarbonate 10-L bioprocess containers.

Catastrophin drug product is prepared as a pre-filled syringe, sterile, preservative-free, clear and colourless isotonic solution, pH 7.0 $\pm$ 0.2, for subcutaneous (sc) administration.
The primary packaging contains:
- a 1 ml siliconised type I glass barrel with a LUER conus
- a grey bromobutyl rubber stopper/ (plunger rod not in contact with the formulation)
- a bromobutyl rubber tip cap

Presentation of the cases

Drug Substance
For MAA three lots of Catastrophin drug substance manufactured by the proposed commercial process have been put on stability.
The bulk drug substance is 0.2 µm filtered, aliquoted into 10L polycarbonate bottles and stored frozen at ≤ -65°C.

The applicant claims a shelf life of 36 months when stored at ≤-65°C.
Stability data are derived from storage at -70°C, -20°C, +5°C and 25°C in 100 ml containers of identical quality as compared to the storage containers.

Upon storage at - 20° C all parameters were within the given specification, at ≤-65°C, all quality parameters are within specifications, except pH, for which considerable OOS results were observed. The applicant confirmed that no other quality parameters indicating product degradation were affected by the drift in pH.

As stability samples for drug substance were shipped on dry ice from the DS manufacturer to the testing laboratory there is some speculation that ingress carbon dioxide from the dry ice might have an effect on pH of the drug substance.

Authorities consider the decrease of pH to be critical.
The company made the proposal to widen the specification limit for pH as the shift does not affect any other stability indicating parameters

Questions:
1. What would you expect to be the regulatory outcome of this issue?
2. What additional information would you require from the applicant?
3. What action could be requested?

Drug Product

At the chosen pH of 7.0, contents of max 6 % non-pegylated and up to 75% deamidated forms are predicted during 2 years of storage at 5°C. At timepoint 0 contents of max. 1.5 % non-pegylated and up to 15% deamidated forms are determined

For the drug product used in the pivotal clinical studies 2.5 – 5% non-pegylated forms and 25-35% deamidation were determined.

The applicant argues that the high level of deamidation neither affects protein structure, functionality or receptor binding properties, nor immunogenicity or the safety profile. Deamidated forms of Catastrophin have been shown in non-clinical studies to be biological active. Thus, it is considered highly improbable that the high level of deamidation had any influence on efficacy. Activity is monitored by a cell based in vitro potency assay and is included in the specification for release and shelf life.

Questions:

1. Is the proposed shelf life of 2 years at 5°C acceptable?
2. Is the application approvable?
3. How would you set the specification?
4. What additional action could be requested from the applicant?

Four years after marketing authorisation the company changes the manufacturing site for the drug product and in addition wants to extend the shelf life to 3 years.

Newly initiated stability studies reveal that at 5°C after 18 months in RP–HPLC (method used for purity) a small peak which elutes prior to the main peak increases from <0.5% to 1.3 % (OOS) . The specification limit for this peak is <1%. The peak is considered to be a product related substance, but was never thoroughly investigated, as previously it was always below the specification limit. Due to the OOS results an investigation was initiated.

To better characterise this peak the company developed a modified and improved RP-HPLC with a better resolution, also eluting for a longer time period. With the newly designed method it turns out that 15 min after elution of the main peak an additional peak (1.5-2 %) appears, that has never been detected before. This peak was not detected with the previous method as the runs were terminated prior to elution.

First investigations indicate that both peaks represent leachables from the bromobutyl stopper of the syringe.

Questions:

1. What would you expect the company to do?
2. What needs to be investigated?
3. What measures might be adequate?

Case study 2

Stability indicating tests: Examplemab

Presentation of the product

The Marketing Authorisation applicant (MAA) has submitted a dossier for a Marketing authorisation application for a biological medicinal product containing Examplemab as active substance.

Examplemab is a fully human monoclonal antibody manufactured form Chinese Hamster Ovary Cells (CHO). Examplemab is an immunomodulatory agent that is being developed for use in the treatment of cancer. The proposed mechanism of action for examplemab is interference of the interaction of Receptor X expressed on a subset of activated lymphocyte cells.

The drug substance is defined as a bulk solution containing examplemab. It is filled in to single-use, pre-sterilized multi-layered high density polyethylene (HDPE) 100-L bioprocess containers.

Examplemab injection is prepared as a sterile, preservative-free, clear and colourless isotonic solution for intravenous (IV) administration. Examplemab is presented as a concentrate solution for infusion at a concentration of 10 mg/ml in two sterile, preservative-free presentations intended for single use: 100 mg/vial (100 mg/10 mL) and 200 mg/vial (200mg/20 mL).

Presentation of the case

Three lots of examplemab drug substance manufactured by the proposed commercial process have undergone comprehensive physicochemical characterization.

The physicochemical characterization, product-related variants and biological activity tests are detailed in the table below.

Physicochemical Characterization of Examplemab

Analysis	Result
Absorptivity	The theoretical UV (280 nm) absorptivity value of examplemab has been calculated to be 1.45 mL mg-1 cm-1. The experimental value was determined to be 1.48 mL mg-1 cm-1.
Amino Acid Sequence	The cDNA-derived amino acid sequence of Examplemab was confirmed by LC-MS/MS sequencing, which includes the variants for the heavy chain amino- and carboxy-terminus, asparagine deamidation, methionine oxidation and N-linked glycosylation. Results from trypsin and supplemental Asp-N peptide maps verify 100% of the cDNA-derived primary sequence of Examplemab.
Amino Acid Analysis	The relative abundance of twelve well recovered amino acids were determined in all three lots and identified by coincidence with NIST standards.
Molecular Weight	The average molecular weight determined by MALDI-TOF is 150,000 Daltons.
Circular Dichroism	The Examplemab CD spectrum has a distinct ellipticity with a minimum in the far-UV at 213 nm to 215 nm, indicating that the main type of secondary structure in the protein is beta-sheet and consistent for an immunoglobulin class of molecules.
Differential Scanning Calorimetry	DSC analysis confirmed that the Examplemab melting temperatures of 67°C, 71°C and 84°C (consistent with an Ig molecule).

Product-Related Variants of Examplemab (post-translational modifications)

Variant Analytical Technique	Results
Amino- (N) Termini Tryptic Peptide Mapping by LC-MS/MS Analysis	The light chain amino-terminal tryptic peptide was determined. The heavy chain contains two amino-termini. The predominant heavy chain amino-terminus was identified as pEPR…..(90.0% to 93.0% abundance) with cyclization of the N terminal glutamine to pyroglutaamte. The unmodified amino-terminus was also present with 7.0% to 10.0% abundance.
Carboxy-(C) Termini Tryptic Peptide Mapping by **LC-MS/MS Analysis**	The light chain carboxy-terminal Asp-N peptide is determined. The predominant heavy chain carboxy-terminus is identified as …G. The unmodified carboxy-terminus (…K) containing the C-terminal lysine (K) is present at lower abundance (0% to 0.5%).
N-linked Glycosylation HPAEC-PAD HPLC-MS Intact ESI-MS	Examplemab has a single N-linked glycosylation site on each heavy chain at Asn296. Three biantennary structures have been identified using HPAEC-PAD and confirmed by MS/MS.
Monosaccharides HPAEC-PAD	The relative abundance of Fucose (15.0% to 17.0%), Galactose (5.0% to 6.0%), Mannose (36.0% to 37.0%) and N-Acetyl Glucosamine (44.0% to 45.0%)
Oxidation LC-MS/MS Peptide Mapping LC/UV Peptide Mapping	Examplemab contains four methionine residues on each heavy chain. No methionine residues are found on the light chains. Met414 has been identified as the most susceptible methionine residue to oxidation. Oxidation levels in three lots were $\leq$ 3%.
Deamidation LC-MS/MS Peptide Mapping LC/UV Peptide Mapping	Examplemab contains 18 asparagine residues on each heavy chain and 4 asparagine residues on each light chain. Four trypsin peptides in the LC-MS/MS peptide map were observed to have detectable levels of asparagine deamidation. Three asparagine residues susceptible to deamidation were found for each heavy chain. One deamidation susceptible asparagine residue was identified on each light chain. The relative percentage of deamidation for all product peaks in three lots was $\leq$ 2.0%. The residues susceptible to deamidation are outside of the variable regions of the heavy and light chains.
Charge Variants Isoelectric Focusing Cation Exchange Chromatography	IEF analysis demonstrates a band pattern of multiple charge isoforms tightly focusing in the pI 7.0 to 8.0 range. The predominant bands focus at pI 7.8. CEX analysis resolves 3 major peaks which have been identified by LC-MS/MS to be the major charge variants. The most abundant charge variant (79% to 81%) in three lots has amino-terminal pyroglutamate at both heavy chains and no carboxy-terminal lysines.
Intra- and Inter-Chain Disulfide Bonds Reduced and Non-Reduced Tryptic Peptide Mapping and Lys-C Peptide Mapping Combined with LC/MS/MS	There are two intra-chain disulfide bonds on each light chain and four intra-chain disulfide bonds on each heavy chain. Examplemab also has one inter-chain disulfide bond between each light chain and heavy chain and two inter chain disulfide bonds between the two heavy chains.
High and Low Molecular Weight Species Reduced and Non-Reduced SDS-PAGE SE-HPLC Analytical Ultracentrifugation	The major band of non-reduced examplemab migrates at approximately 225 kDa as compared with standards. The two major bands of reduced examplemab migrate at approximately 50 kDa and 30 kDa. Minor bands are also present just below the major band. The predominant examplemab species exists in the monomeric form with dimer being the predominant high molecular weight form, detected at very low levels.
Molecular Weight SE-MALS	The molecular weight was determined to be 150 kDa to 151 kDa for three lots. No High Molecular Weight species were detected.

Biological Activity of Examplemab

Biological Activity Analytical Technique	Result
Binding to Receptor X Activity ELISA	Examplemab binds specifically to ReceptorX/Fc that is coated on to the surface of microtiter plate wells. Results demonstrate that binding to Receptor X relative to the reference standard is comparable for the three lots (90% to 95%).
Potency Competition ELISA	In competition with a protein Y and the Fc region of human IgG, examplemab binds to receptor X. The potency for three lots relative to the reference standard was determined to be 101% to 105%.
Kinetics of Binding to RECEPTOR X Surface Plasmon Resonance	Kinetic properties of three examplemab lots were comparable for binding to covalently immobilized Receptor X .
Binding to Cell-Surface Expressed RECEPTOR X Fluorescence Activated Cell	Receptor X -expressing hybridoma cells were incubated with fluorescent labeled protein and varying concentrations of examplemab. The loss of signal is directly proportional to the binding of examplemab to hybridoma cells. The binding characteristics for the three lots are similar.
Fc-Receptor Binding ELISA	The average 50% effective concentration was determined.
ADCC and CDC ADCC and CDC In vitro Assays	Examplemab did not elicit CDC and demonstrated moderate activity in an in vitro antibody-dependent cytotoxicity assay. The measurable but variable levels of ADCC were unrelated to the level of RECEPTOR X expression.

Summary:

Examplemab drug substance contains product-related variants that contain modifications at the amino- and carboxy-termini, N-linked glycoforms, deamidated and oxidized amino acids, charge variants, and high and low molecular weight species. These variants are typical of monoclonal antibodies produced by mammalian cell culture and are not expected to affect overall in vivo biological activity. Some of these variants (deamidated or oxidized) are considered as stability indicating.

Questions:

1. Which tests used in the characterisation of the active substance would you choose for inclusion in you stability program for an active substance?
2. What additional tests would you include in the stability studies for the drug substance?
3. What additional tests would you include in the stability studies for the drug product?

Case study 3

Stability issues: "recombinant Protein X"

Presentation of the product

Protein X is a cytokine manufactured by recombinant DNA technology that contains 170 aminoacids. Protein X is used in the treatment of leukaemia.

Recombinant protein is a ready-to-use solution for injection in a multidose pen available in three strengths. It is approved for subcutaneous administration.

The drug product is manufactured in three available strengths. Its formulation contains m-cresol as preservative and EDTA.

The product is marketed in multidose pens. The primary packaging contains:
-a carpoule: Type 1, glass carpoule
-a plunger: grey bromobutyl rubber plunger
-a seal: combiseal, aluminium with grey halobutyl rubber disc

Presentation of the case

Protein X has been marketed for more than 5 years. At the initial application, a shelf life of 15 months at 2-8°C was approved. This shelf life was approved based on supportive stability data on 3 representative batches. It was confirmed with post approval stability studies with 3 commercial batches.

The stability indicating tests during the stability studies were:

- Description
- pH
- Assay HPLC
- Potency Assay
- Purity: SDS-PAGE (Reducing), SDS-PAGE (Non-Reducing), Isoelectric Focusing
- m-Cresol Assay
- Particulate Matter
- Sterility

The Marketing Authorisation Holder (MAH) requested by a variation to increase the shelf life from 15 months to 18 months at 2-8°C. The applicant provided:

- Long-term stability study for 9 commercial batches of Product X Multidose Pen (3 batches for each Strength A, B and C)) stored at 2-8°C for up to the proposed shelf life of 18 months. (over 30 individual multidose pens used)

- Short term/high temperature study for 3 commercial batches of Product X Multidose Pen (1 batch for each Strength A, B and C) at the 18-month test interval with exposure to 30°C for 2 or 5 days.

- Patient Use Test study for 3 commercial batches of Product X Multidose Pen (1 batch for each Strength A, B and C) at the 18-month test interval with 12 x 4 hour exposures to 25°C over 4 weeks.

All batches met the specifications in the stability studies and an extension of the shelf life of 18 months at 2-8°C was approved.

6 months later the extension of shelf life was approved, the MAH submitted the following data:

Testing of one sample batch B1 at the 18 month stability time point for Protein X Multidose Pen, generated an Out Of Specification (OOS) result for HPLC Assay % Protein Target. The results reveal low protein content in the tested sample. These results can be found in table 1.

Questions:

1. What additional information would you request from this result?
2. What actions would you consider?
3. What would you expect to be the regulatory outcome of this issue?

Table: Results of the long term stability study on Batch B at 2-8°C

		Refrigeration (5±3°C)						
Samples	Test	Specification	Stability Test Interval (Months)					
			Initial	6	9	12	15	18
	Description	Clear, colourless (as defined in Ph. Eur 2.2.2) solution, essentially free of visible particles	Complies	Complies	Complies	Complies	Complies	Complies
All samples	pH	6.0 - 7.0 pH Units	Complies	Complies	Complies	Complies	Complies	Complies
Sample 1 Batch B	HPLC % Target Protein	80 – 115%	99	99	95	90	90	37,7
Sample 2 Batch B		80 – 115%	106	104	104	103	100	99,5
Sample 3 Batch B		80 – 115%	106	103	103	102	100	100,6
All samples	Potency Activity % Label Strength	90 – 110%	Complies	Complies	Complies	Complies	Complies	Complies
All samples	SDS-PAGE (R) % Total Impurities	≤ 5%	Complies	Complies	Complies	Complies	Complies	Complies
All samples	SDS-PAGE (NR) % Total Impurities	≤ 5%	Complies	Complies	Complies	Complies	Complies	Complies
All samples	IEF % Desamido Species	≤ 15%	Complies	Complies	Complies	Complies	Complies	Complies
All samples	m-Cresol Assay % of Labelled Amount	90 – 105%	Complies	Complies	Complies	Complies	Complies	Complies
All samples	Particulate Matter No of particles/carpoule ≥10µm	Meets the current Ph. Eur specifications for small volume parenterals	Complies	Complies	Complies	Complies	Complies	Complies
All samples	Sterility	Complies with Ph. Eur.	Complies	---	---	Complies	Complies	Complies

Case study 4

In-use stability: flu vaccine X multidose vials

Presentation of the product:

Flu vaccine X is a suspension for injection that contains parts ('surface antigens') of the different subtype of the influenza (flu) virus.

Flu vaccine X is formulated with an adjuvant (o/w emulsion) and is presented in 10mL multi-dose vials. Thiomersal is included in the formulation as preservative.

A 10 ml multidose vial is intended for 10 doses (1 mL/dose)

Flu vaccine X has been authorised more than 1 year ago. A shelf life of 12 months at 2-8°C was approved based on supportive data on representative batches. Data generated by commercial batches in the post-approval phase confirmed this shelf life.

Presentation of the case:

The company submits a variation claiming an "in use" shelf life of 14 days.

The company proposed the following stability study:

The stability study will have 2 arms:

a) In-Use test performed at the beginning of the shelf life (time zero) -> Two batches will be tested
A total amount of 200 samples, including a 20% of back-up, is needed for each batch.

b) In-Use test performed at the end of the shelf life (the 11th month) -> One batch will be tested
A total amount of 250 samples, including a 20% of back-up, is needed for the batch.

Dose withdrawal schedule:

In-Use test performed at time zero.

Timepoints	No. of doses taken out	Remaining doses after removal
Time zero	1 x 1 mL doses	9 x 1 mL doses
6 hours	1 x 1 mL doses	8 x 1 mL doses
24 hours	1 x 1 mL doses	7 x 1 mL doses
2 days	1 x 1 mL doses	6 x 1 mL doses
7 days	1 x 1 mL doses	5 x 1 mL doses
14 days	1 x 1 mL doses	4 x 1 mL doses
28 days	2 x 1 mL doses	2 x 1 mL doses

In use test performed at month 11 will follow the same study design of dose withdrawal.

The necessary amount of samples will be allowed to reach room temperature (approx 25°C) (in about 30 minutes) and then some doses will be taken out. After the removal of the doses, sufficient samples will be sent to QC labs for the relevant analyses and the remaining samples will be put again at 2°-8°C to continue the study, where relevant.

The proposed testing plan is detailed in the table below

Table 3-3 Testing plan for the In-Use test performed at time zero and at the time of 11 months.

Analysis	Timepoints						
	0	6 hours	24 hours	2 days	7 days	14 days	28 days
HA identity and titre by SRID	X	-	-	-	-	X	X
Appearance	X	-	-	-	-	X	X
pH	X	-	-	-	-	X	X
Adjuvant content and identity	X	-	-	-	-	X	X
Endotoxin	X	-			-	X	X
Sterility	X*	X*	X*	X*	X*	X*	X*

X: planned test - test not planned

* After withdrawal of the doses, sterility will be performed on the remaining quantity of vaccine in the single container as this is an in use testing and not a release testing.

The company has provided adequate description of the testing methods and the specifications. Methods have been validated.

Questions:

The company designed the protocol to support an 'in-use' shelf life of 28 days.

1. Is the testing plan proposed in the stability protocol adequate?
2. Is the proposed number of batches adequate for the study?
3. Is the dose withdrawals plan adequate?
4. The applicant submits this variation with real time data at day 14 of the first arm of the study is concluded and applies for 14 days shelf life. Would this be acceptable? Would there be any additional document to be provided by the applicant?