EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

03 August 2012
EMA/488088/2013
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

MabThera

Rituximab

Procedure No.: EMEA/H/C/165/A-20/0078

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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1. Background information on the procedure

The European Medicines Agency and the European Commission were informed by the marketing authorisation holder (MAH) of MabThera about the unexpected presence of *Leptospira licerasiae* in seed-train bioreactors used for the production of the active substance, rituximab, at its Genentech Vacaville site (further referred to as Vacaville in this report), located in the United States of America (USA)[1]. *Leptospira licerasiae* was only detected at pre-harvest phase (a very early stage of manufacturing) by microscopic visual examination and all cultures confirmed to be contaminated were discarded. A further in-depth analysis of the matter was deemed necessary to evaluate the impact of the quality issues identified in the manufacturing of rituximab on the quality and safety of the finished product.

In view of the above the European Commission initiated a procedure under Article 20 of Regulation (EC) No 726/2004. The European Commission requested the CHMP on 15 December 2011 to assess the above concerns and its impact on the benefit/risk for MabThera, and to give its opinion on whether the marketing authorisation for this product should be maintained, varied, suspended or withdrawn.

2. Scientific discussion

MabThera (rituximab), authorised since 2 June 1998, is a genetically engineered chimeric mouse/human monoclonal antibody indicated for use in adults for non-Hodgkin's lymphoma, chronic lymphocytic leukaemia (CLL) and rheumatoid arthritis[2].

In May 2011, the presence of *Leptospira licerasiae* (also referred to as Leptospira in this document) was detected at very early stages of manufacturing (pre-harvest) in seven rituximab seed-train bioreactors at the Vacaville (VV) site. The organism had never been detected before in the Roche/Genentech biological production network and it was identified as *Leptospira licerasiae* by 16S DNA sequencing. The only indication of contamination was microscopic visual examination. All pre-harvest cultures confirmed to be contaminated were discarded. The investigation at the time considered this an isolated event, however, in August 2011, a second contamination of the same organism occurred also at very early stages of manufacturing (pre-harvest) in one bioreactor. All pre-harvest cultures confirmed to be contaminated were discarded. A sample with the organism was successfully cultured allowing additional studies to understand root cause and potential additional detection controls. Drug substance lots were not visually contaminated, but all those linked through genealogy to the contaminated cultures were not used but considered for further investigations.

In December 2011 the Committee for Medicinal Products for Human Use (CHMP) was informed of the issues observed at the Vacaville site. *Leptospira licerasiae* had been detected at pre-harvest stage but the drug substance batches produced did not confirm the presence of Leptospira by microscopic visual examination and all cultures confirmed to be contaminated were discarded. Downstream in-process controls and certificates of analysis met acceptance criteria and specific conditions such as low pH and the downstream processing aimed to provide a robust clearance of process-related impurities and potential process contaminants. However, a further in-depth analysis of the matter was deemed necessary to evaluate the impact of the quality issues identified in the manufacturing of rituximab on the quality and safety of the finished product.

The MAH provided relevant information on the root cause evaluation and prevention of future Leptospira problems, and these were considered in the framework of this review. An inspection was held at the Vacaville site in February 2012, and its results were also taken into account. A summary of the relevant data for this review is presented herein after.

[1] Two sites are currently approved for the manufacture of MabThera's active substance (rituximab), Genentech Vacaville and Genentech Oceanside, both located in the United States of America (USA).
[2] For the full indication please refer to the product information which can be found on the EPAR for MabThera.

2.1. Quality aspects

2.1.1. Root cause

Further to the events at the Vacaville site, a methodological investigation into the root cause of the detection of *Leptospira licerasiae* was initiated. The Kepner Tregoe (KT) Problem Analysis process was utilised to evaluate possible causes of the contamination detected at pre-harvest level. Investigations considered possible root causes, including cell culture media components and solution preparation processes; cell banks used in Vacaville; breaches in the cooling tower water; personnel; antifoam; pest control (Leptospira introduced by a pest or small mammal as a carrier); insufficient bioreactor cleaning and autoclaving; carbonate (added to media); and the water for injection (WFI) system.

Based on all data provided, the most likely cause of the presence of *Leptospira licerasiae* was the contamination of the fermentation process of rituximab, at the Small Volume Media Preparation (SVMP) area where media preparation took place in open plastic vessels in a specific (air) classified area. A combination of personnel (as external carriers) and/or these operations in open plastic vessels was considered to have created a plausible pathway for the organism to be introduced into the media. Spirochetes from the Leptospiracae family were also observed in the environment external to the Vacaville facility ([redacted] Irrigation [redacted] water). [redacted] was an untreated open water source that was intermittently used as make-up water to the cooling tower; its use has now been officially discontinued. Other possible root causes were considered, such as the contamination of rituximab's working cell banks, however based on available evidence, including negative PCR (polymerase chain reaction) results for potential Leptospira DNA and negative testing for growth of Leptospira, the likelihood of these being contaminated was considered very low.

2.1.2. Corrective actions

Based on the combination of factors which led to the findings of *Leptospira licerasiae* at the Vacaville production site at pre-harvest level, considerations were given to the existing processes, including downstream elimination, and any need for improvements in the manufacturing process.

An assessment of the rituximab purification process inactivation and clearance capability for Leptospira and Leptospira-derived contaminants was undertaken. In addition the capability of the rituximab viral filtration step to remove Leptospira was assessed at small scale. Furthermore, the capability of the equipment sanitation procedures was evaluated.
The [redacted] rituximab purification process provide robust clearance of a broad spectrum of process related impurities and process contaminants with variable biochemical and physical characteristics. The demonstrated ability to clear [redacted] impurities to acceptable levels provides assurance that Leptospira and Leptospira-derived contaminants would likely be removed from the rituximab product. The product pool is also subject to a low pH hold step for putative retrovirus inactivation. In-house testing showed these conditions are capable of inactivating *Leptospira licerasiae*. In addition, no Leptospira was detected downstream of the filter when challenged, in a small scale study, with approximately 4.62×10^8 to 4.95×10^8 Leptospira/cm^2 of filter surface area, demonstrating robust removal of Leptospira. Furthermore, based on the data provided, the existing regeneration and sanitisation conditions in use at the site are expected to inactivate any potential *Leptospira licerasiae*.

Regardless of the capacity of the manufacturing process to eliminate any existing Leptospira, an additional risk assessment was performed to evaluate the adequacy of current microbial control systems in drug substance production for preventing microbial contamination and evaluate the current detection and contamination response procedure. Interim corrective and preventive actions (CAPA) were initiated to prevent contamination at pre-harvest level and, if contamination occurs, ensure its detection. Measures included elimination of the use of plastic containers in the preparation of small volume cell culture media and replacement with an existing stainless steel media tank; the use of High Temperature Short Time (HTST) treatment of cell culture media to different stages in the cell culture process; implementation of microscopic visual examination (MVE) for all CHO-derived products; EMJH (Ellinghausen-McCullough-Johnson-Harris) medium culture method testing on non-frozen pre-harvest cell culture fluid samples for all CHO-derived products sharing the same media components or cell source as rituximab; PCR testing on pre-harvest cell culture fluid samples (fresh or frozen) for all CHO derived products sharing the same media components or cell source as rituximab; and EMJH medium

culture method testing on residual fluid from each rituximab Working Cell Bank (WCB) ampoule following each thaw operation.

The CHMP considered that the proposed enhancements were adequate in preventing the occurrence of future Leptospira contaminations. In addition, the measures in place will ensure the detection of Leptospira, if a contamination would re-occur. A period of monitoring and assessment of the measures in place is foreseen, and reports are expected to be submitted by the MAH latest by January 2013. During this period, the interim CAPA will continue. If any changes or discontinuation of the agreed interim measures implemented at the site is envisaged, this shall be immediately reported to the CHMP for assessment. A more sensitive PCR method is also being developed by the MAH and will need to be validated. Its completion is expected by end December 2012.

All changes to processes, frequencies or limits (e.g. specifications) resulting from the evaluation at the site should be submitted to the European Medicines Agency/CHMP, through the appropriate variations.

2.1.3. Inspection

The CHMP requested the inspection of the active substance manufacturing site located in Vacaville, USA, to investigate the issue and the proposed CAPA measures *in loco*. The inspection took place on 7-9 February 2012. The inspection involved a thorough assessment of laboratories, warehouses as well as manufacturing and utility facilities. The inspectors also evaluated the quality management systems at the site. The inspectors considered that overall the root cause investigation conducted was solid and the site had good understanding of GMP, and rules were generally followed. A few minor issues were reported at the site; these will be followed by the inspectorate for completeness but bare no concern for this review.

2.2. Clinical aspects

2.2.1. Clinical safety

Leptospira licerasiae is a bacterial species that can cause Leptospirosis, a water-borne disease transmitted from animals to humans. Leptospires are thin and have a typical helical shape; this unique morphology makes them easily recognised under the microscope. A multi-layered Gram negative like outer membrane or envelope surrounds the cell. It contains proteins such as lipopolysaccharide (endotoxin), lipoproteins and transmembrane outer membrane proteins. These components as well as other proteins such as exotoxins (hemolysins) have been identified as candidate virulence factors which may contribute to Leptospira infection and disease.

Based on the available data, no viable Leptospira were detected at drug substance or finished product levels. The assessment of rituximab's purification process capacity for removal of a variety of proteins and process-related impurities supported that it would be capable of removing Leptospira and Leptospira-derived contaminants (endotoxins and exotoxins). In addition, even if Leptospira contaminated cell culture had been processed through the rituximab purification process, the manufacturing conditions employed as well as equipment cleaning are considered to be sufficient to inactivate Leptospira. Rabbit pyrogen test results were also provided showing that all tested finished product and drug substance lots met the requirements for non pyrogenicity, including finished product batches derived from pre-harvest cell culture which tested PCR-positive (i.e., drug product batches which were processed from Leptospira containing pre-harvest cell culture, but which were not used).

The detection of *Leptospira licerasiae* at pre-harvest level has been addressed by introduction of interim corrective and preventive actions (see also section 2.1.2 above), and all pre-harvest cultures considered to be contaminated were discarded (will not be further processed). Although it is noted that pre-harvest media which tested positive to *Leptospira licerasiae* will not be used for production, the current rituximab processing would inactivate any bacteria and remove its endotoxins and exotoxins.

It was concluded that the treatment with rituximab finished product from drug substance batches produced at Vacaville was not associated with clinically relevant risks for patients.

2.3. Product information

There were no changes to the product information.

3. Overall discussion and benefit/risk assessment

MabThera (rituximab), authorised since 2 June 1998, is a genetically engineered chimeric mouse/human monoclonal antibody indicated for use in adults for non-Hodgkin's lymphoma, chronic lymphocytic leukaemia (CLL) and rheumatoid arthritis[3].

In May 2011, the presence of *Leptospira licerasiae* was detected at very early stages of manufacturing (pre-harvest) in seven rituximab seed-train bioreactors at one of the active substance's manufacturing site located in Vacaville, United States of America. The only indication of contamination was microscopic visual examination. All pre-harvest cultures confirmed to be contaminated were discarded. The investigation at the time considered this an isolated event however, in August 2011, a second contamination of the same organism occurred also at very early stages of manufacturing (pre-harvest) in one bioreactor. All pre-harvest cultures confirmed to be contaminated were discarded. A sample with the organism was successfully cultured allowing additional studies to understand root cause and potential additional detection controls. Drug substance lots were not visually contaminated, but all those linked through genealogy to the contaminated cultures were not used but considered for further investigations.
Leptospira licerasiae is a bacterial species that can cause Leptospirosis, a water-borne disease transmitted from animals to humans. However, based on the investigations the CHMP considered that treatment with rituximab finished product from drug substance batches produced at Vacaville was not associated with clinically relevant risks for patients.
The bacteria was only detected at very early stages of manufacturing (pre-harvest level), and no Leptospira were detected at drug substance or finished product levels. Furthermore, even if cultures where Leptospira or Leptospira-derived contaminants present at pre-harvest level had been used, the current rituximab purification process for proteins and process-related impurities supported that the process would be capable of removing them. The conditions of manufacture and cleaning regimen would also be expected to inactivate any Leptospira.

Regarding the quality investigations, and based on all available quality data, including information from an inspection held at the Vacaville site, the most likely root cause for the contamination of the rituximab seed train bioreactors with *Leptospira licerasiae* was the media preparation operations in the Small Volume Media Preparation (SVMP) area. A combination of personnel (as external carriers) and/or operations in open plastic vessels was considered to have created a plausible pathway for the organism to be introduced into the media. The CHMP concluded that batches of drug substance linked to cultures which tested positive at pre-harvest will not be further processed and interim corrective and preventive measures (CAPA) were introduced at the site to minimise any potential contamination and maximise the detection of Leptospira. The measures included elimination of the use of plastic containers in the preparation of small volume cell culture media and replacement with an existing stainless steel media tank; the use of High Temperature Short Time (HTST) treatment of cell culture media to different stages in the cell culture process; implementation of microscopic visual examination (MVE) for all CHO (Chinese hamster ovary)-derived products; EMJH (Ellinghausen-McCullough-Johnson-Harris) medium culture method testing on non-frozen pre-harvest cell culture fluid samples for all CHO-derived products sharing the same media components or cell source as rituximab; PCR (polymerase chain reaction) testing of Leptospira DNA on pre-harvest cell culture fluid samples (fresh or frozen) for all CHO derived products sharing the same media components or cell source as rituximab; and EMJH medium culture method testing on residual fluid from each rituximab Working Cell Bank (WCB) ampoule.

The CHMP considered that the proposed measures were adequate in preventing the occurrence of future Leptospira contaminations. In addition, the measures in place will ensure the detection of Lepstopira, if a contamination would re-occur. A period of monitoring and assessment of the measures in place is foreseen, and reports are expected to be submitted by the marketing authorisation holder (MAH) latest by end January 2013. During this period, the interim CAPA will continue. If any changes or discontinuation is envisaged, this shall be immediately reported to the CHMP for assessment. A

[3] For the full indication please refer to the product information which can be found on the EPAR for Mabthera.

more sensitive PCR method is also being developed by the MAH and will need to be validated. Its completion is expected by end of 2012.

Benefit/risk balance
In view of the above, the Committee considers that the benefit risk balance of MabThera (rituximab) is positive under normal conditions of use, subject to agreed conditions.

4. Overall conclusion

Having considered the data provided by the MAHs in writing and in the oral explanations, and the information available from the inspection of the affected site, the CHMP concluded that the data are sufficient to conclude on the Leptospira findings at the manufacturing site located in Vacaville, USA.

Therefore the CHMP recommended the maintenance of the marketing authorisation subjected to the following conditions:

(i) The MAH shall not use any drug substance batch manufactured from Leptospira PCR positive pre-harvests;

(ii) The MAH shall submit all on-going CAPA assessments by end January 2013;

(iii) The MAH shall develop and validate a more sensitive PCR method of detection of Leptospira and submit its report by end 2012;

(iv) The MAH shall submit the appropriate variations if changes to processes, frequencies or limits resulting from the evaluation at the site are to be implemented.

The Committee considers that the benefit risk balance of MabThera (rituximab) is positive under normal conditions of use, subject to agreed conditions.

5. Conclusion and grounds for the recommendation

- The Committee considered the procedure under Article 20 of Regulation (EC) No 726/2004, for MabThera initiated by the European Commission;

- The Committee reviewed all available data provided by the MAH orally and in writing regarding the detection of *Leptospira licerasiae* at very early stages of manufacturing (pre-harvest) of the active substance at a site located in Vacaville, USA; the Committee considered also the information available from the requested inspection of the site, held in February 2012;

- The Committee considered that the most likely root cause of the contamination has been identified as the media preparation operations in the Small Volume Media Preparation (SVMP) area. Adequate corrective and preventive measures were introduced at the Vacaville site to minimise any potential contamination and maximise the detection of Leptospira;

- The Committee concluded that the findings are not associated with any clinically relevant risk for patients treated with MabThera; Drug Substance batches linked to pre-harvests that tested positive for Leptospira will not be further processed, and no bacteria were detected at drug substance or finished product levels. In addition, the CHMP considered that the robustness of the manufacturing process should ensure removal of any Leptospira or Leptospira-derived contaminants;

- The Committee concluded that the benefit-risk balance of MabThera is positive under normal conditions of use, subject to the conditions agreed.

The CHMP has therefore recommended the maintenance of the marketing authorisation, subject to the conditions laid down in Annex II of the opinion.