



MEMORANDUM

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

To: File (STN 125351/0) and Jie He, OBRR/DBA/RPMB

From: Natalya Ananyeva, Ph.D., Visiting Scientist, Laboratory of Hemostasis (LH), Division of Hematology (DH)/OBRR

Through: Timothy Lee, Ph.D., Acting Chief, LH/DH/OBRR

Subject: Final CMC Review of Nycomed’s BLA 125351/0 for Fibrin Sealant Patch (TachoSil)

This memorandum summarizes the Final Review of an original Biologics License Application (STN 125351/0) for Fibrin Sealant Patch (FDA-recommended name) submitted by Nycomed, Danmark ApS. The proposed trade name is TachoSil. TachoSil is a combination product where two Active Substances – Human Fibrinogen and Human Thrombin – are coated onto an Equine Collagen Sponge (classified as Medical Device) to facilitate their application to the wound area in clinical practice. Human Fibrinogen Active Substance and Human Thrombin Active Substance are supplied by -----(b)(4)-----

All three BLA’s are being reviewed in parallel. Nycomed claims the clinical indication for Fibrin Sealant Patch *as an adjunct to hemostasis in cardiovascular surgery when control of bleeding by standard surgical techniques, such as suture, ligature or cautery, is ineffective or impractical.*

BACKGROUND

Fibrin glues have a long history of therapeutical use for achieving hemostasis and tissue sealing during surgical interventions. In first-generation fibrin glues, Fibrinogen and Thrombin components are kept separately in -----(b)(4)----- . For use, solutions are prepared and mixed during application on the wound surface. The main drawback of liquid fibrin glues is that they can be washed away before sufficient polymerization of fibrin has occurred. Next generation of fibrin glues included application of liquid components to absorbable carriers (collagen fleece) but the preparation is cumbersome.

Fibrin Sealant Patch (TachoSil) from Nycomed, Denmark, is a ready-to-use, degradable fibrin sealant product developed for topical use. TachoSil consists of two Active Substances - Human Fibrinogen and Human Thrombin – coated onto a Collagen Sponge of equine origin. The Collagen Sponge serves as a flexible and mechanically-stable carrier for the Active Substances to ensure their in-place position. Riboflavin is present as a yellow colorant to indicate the active

side of the patch. Upon contact with blood, other body fluids or saline solution, the coagulation factors in the TachoSil Patch are hydrated, and the subsequent fibrinogen-thrombin reaction initiates the last step of the coagulation cascade – formation of a viscous and elastic fibrin clot. Upon moistening, the Patch becomes pliable and extensible to be molded to the affected tissue surface and to accommodate for the physiological movements of tissues and organs.

TachoSil is the third-generation Fibrin Sealant products with its predecessors TachoComb (which used bovine thrombin and bovine aprotinin as an antifibrinolytic agent) and TachoComb H (human thrombin but bovine aprotinin). TachoComb was first marketed in Austria and Germany in 1991/1992. TachoComb H was marketed in Germany and Austria from 2001 – 2005. Compared to the predecessor products, TachoSil is free of bovine components as both Active Substances are of human origin and does not contain bovine aprotinin. Aprotinin was removed from the formulation because it was shown not to affect fibrinolysis with the use of TachoComb and TachoComb H.

TachoSil was approved in Europe in June, 2004. In February 2009, the European Commission approved the extended scope of TachoSil indications “for supportive treatment in surgery for improvement of hemostasis, to promote tissue sealing, and for suture support in vascular surgery where standard techniques are insufficient”. More than 400,000 patients have been treated in the European Union with TachoSil since June 2004.

The only difference between the European and US TachoSil products is that the active substances Human Fibrinogen and Human Thrombin contained in TachoSil for the US market are manufactured from human plasma derived from FDA-licensed centers only. TachoSil is produced in three sizes: Standard (9.5 cm x 4.8 cm = 45.6 cm²), Midi (4.8 cm x 4.8 cm = 23.0 cm²) and Mini (3.0 cm x 2.5 cm = 7.5 cm²).

At present, the following Fibrin Sealant products (as frozen solutions and lyophilized powder) are licensed by FDA: TISSEEL (Baxter Healthcare Corp.), ARTISS (Baxter Healthcare Corp.), CROSSEAL and EVICEL (OMRIX Biopharmaceuticals). TachoSil will be the first FDA-licensed Fibrin Sealant Patch.

REVIEW HISTORY

Nycomed submitted the original BLA for TachoSil on 5 June 2009 with the following two proposed indications as:

- (1) -----(b)(4)-----
- (2) An adjunct to hemostasis in cardiovascular surgery

At the mid-cycle of the review process, a number of issues were identified by the clinical and statistical reviewers regarding the proposed indication #1: -----(b)(4)-----
-----; there were issues related to the clinical trial design; risk-to-benefit assessment; and statistical issues (bringing to question the significance of the reported difference in primary efficacy).

An Information Request was sent to the Sponsor on 25 November 2009. Nycomed submitted their responses on 21 December 2009 which were mainly based on post-hoc analyses and did not convincingly demonstrate the link between the primary efficacy of TachoSil and direct clinical benefits to the patient for indication #1. During the Teleconference on 22 January 2010, the Division informed Nycomed that the submission did not support approval of the (b)(4)-indication and suggested two administrative options – (i) Complete Response letter for the entire BLA or (ii) decoupling of the two indications, i.e., -----(b)(4)-----, but continuing review of indication #2 as An Adjunct to Hemostasis in Cardiovascular Surgery. -----(b)(4)----- and proceed with the hemostatic indication only.

Composition of TachoSil Fibrin Sealant Patch

| Ingredient | Wt/cm ² Unit/cm ² Sponge | Standard Size | Midi Size | Mini Size | Function | Reference to Standard |
|---|--|------------------|-----------|-----------|------------------------|--------------------------------------|
| Active Ingredients | | | | | | |
| Fibrinogen Active Substance (b)(4) | | | | | | |
| Human Fibrinogen | 5.5 mg | 250.8 mg | 126.5 mg | 41.3 mg | Active Ingredient | In-House (linked to Internat.) |
| Albumin | -(b)(4)- | -(b)(4)- | -(b)(4)- | -(b)(4)- | Stabilizer | |
| Sodium chloride | -(b)(4)- | -(b)(4)- | -(b)(4)- | -(b)(4)- | Excipients | |
| Sodium citrate | -(b)(4)- | -(b)(4)- | -(b)(4)- | -(b)(4)- | | |
| L-arginine hydrochloride | -(b)(4)- | -(b)(4)- | -(b)(4)- | -(b)(4)- | | |
| Human Thrombin | | | | | | |
| Human Thrombin | 2.0 IU | 91.2 IU | 46.0 IU | 15.0 IU | Active Ingredient | In-House (linked to Internat.) |
| Sodium chloride | -(b)(4)- | -(b)(4)- | -(b)(4)- | -(b)(4)- | Excipients | |
| Sodium citrate | -(b)(4)- | -(b)(4)- | -(b)(4)- | -(b)(4)- | | |
| Other Ingredients | | | | | | |
| Riboflavin | -(b)(4)- | -(b)(4)- | -(b)(4)- | -(b)(4)- | colorant | -(b)(4)- |
| ----(b)(4)---- ----- | -(b)(4)- | -(b)(4)- | -(b)(4)- | -(b)(4)- | ---(b)(4)-- ----- | -(b)(4)- |
| ---(b)(4)-- ----- | -(b)(4)- | -(b)(4)- | -(b)(4)- | -(b)(4)- | ---(b)(4)-- ----- | -(b)(4)- |
| Equine Collagen Sponge | -(b)(4)- | -(b)(4)- | -(b)(4)- | -(b)(4)- | Mechanic al support | -(b)(4)-- |

Optimal concentrations of Active Substances - Fibrinogen and Thrombin – were determined in two animal models as judged by the -----(b)(4)-----

SUMMARY OF REVIEW

I. MANUFACTURING SITES

| Name and Address | Responsibility |
|--|--|
| NYCOMED Austria GmbH, St. Peter Strasse 25, A-4020 Linz, Austria (Building 33) | Manufacture and packaging of TachoSil |
| ----- (b)(4) ----- ----- ----- | ----- (b)(4) ----- |
| NYCOMED Austria GmbH | Analytical testing (in-process, release, stability) except for Sterility, Pyrogen and General Safety testing |
| ----- (b)(4) ----- ----- ----- | ----- (b)(4) ----- |
| ----- (b)(4) ----- ----- | ----- (b)(4) ----- |

II. MANUFACTURE

The Manufacture of TachoSil Final Product includes the production of the Equine Collagen Sponge and the production of TachoSil Fibrin Sealant Patch, i.e., coating the collagen sponge sheets with a suspension of the Active Substances – Human Fibrinogen and Human Thrombin.

3.2.P.2.1. Collagen Sponge Process Development

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

Seven (7) Pages Determined to be Non-Releasable: (b)(4)

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

Reviewer's comment: The changes in the manufacture of TachoSil are justified by validation studies and analytical release testing.

III. CONTAINER CLOSURE INTEGRITY

Considering the hygroscopic properties of TachoSil, Nycomed provides a container closure system for TachoSil that consists of primary and secondary packaging. This double packaging system is used to protect the product against humidity, light, microbial ingress, foreign particles, and to maintain sterility after gamma irradiation, prior to patient use. The primary and secondary packaging for TachoSil is sized on the dedicated packaging line to fit each of the three TachoSil sizes (standard, midi, mini). The primary package is an inner blister pack with a single TachoSil sheet which can be of standard, midi or mini size. The inner or primary packaging manufactured by ----- (b)(4) -----, is a blister pack with a deep drawn tray made of gold lacquer polystyrene foil contact, sealed with a grid varnish coated (- (b)(4) -) medicinal paper on top. A vapor-proof secondary container is made of aluminum laminate foil. Sterilization of TachoSil is performed by gamma irradiation in the secondary packaging.

IV. STERILIZATION BY GAMMA IRRADIATION

TachoSil final product is sterilized by gamma irradiation using gamma-rays from a ---- (b)(4) ---- based on the following:

----- (b)(4) -----

Gamma irradiation is performed with ----- (b)(4) -----

----- (b)(4) ----- . Thus, the actual dose is ----- (b)(4) ----- . The

sterilization method and the sterilization dose have been used for the predecessor products - TachoComb, TachoComb H, TachoSil in Europe - for more than 15 years and not a single case of non-sterility has been observed.

Validation of Sterilization Process

The irradiation sterilization process of TachoSil was validated in two validation studies (Document 0903X-VB-0000008.01):

- A base validation study in 2008
- A re-validation study (auditing of sterilization dose of -(b)(4)-) in 2009

The validation included:

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

----- (b)(4) -----

------(b)(4)-----

Thus, validation studies proved that the irradiation process is suitable for TachoSil and yields a sterile product with SAL of -(b)(4)- as required in ----(b)(4)----.

V. ASSESSMENT OF COMPARABILITY OF TACHOSIL EU AND TACHOSIL INTENDED FOR THE US MARKET

Comparative investigations were performed with the Active Substances – Human Fibrinogen and Human Thrombin, with the Coating Suspensions, as well as with the TachoSil products before (TachoSil IP) and after gamma irradiation (TachoSil Bulk).

------(b)(4)-----

Purity/Identity

------(b)(4)----- Analysis

Comparative ----(b)(4)--- demonstrated no differences in the -----(b)(4)----- between “EU” and “US” lots of Human Fibrinogen and Human Thrombin. Comparison of TachoSil “IP” and “Bulk” showed that -----(b)(4)-----

fragments (mainly from -----(b)(4)-----) are more visible (see photos below).

Three (3) Pages Determined to be Non-Releasable: (b)(4)

-----~~(b)(4)~~-----

-----~~(b)(4)~~-----

-----~~(b)(4)~~-----

Reviewer's comment: The biocomparability program demonstrated that the adaptations of Active Substances in the TachoSil US as compared to the commercial TachoSil EU did not affect the identity, purity, or potency of the final drug product.

VI. CONTROL OF STARTING MATERIAL

The Active Substances - Human Fibrinogen and Human Thrombin - contained in TachoSil US are supplied by -----~~(b)(4)~~-----

Both Active Substances are manufactured from human plasma collected at FDA-licensed centers in the US. Both Human Fibrinogen and Human Thrombin are bioburden controlled - NMT ----~~(b)(4)~~---- for Thrombin and NMT ---~~(b)(4)~~--- for Fibrinogen. In addition, -----~~(b)(4)~~-----
and these results are also applicable for ~~(b)(4)~~- Fibrinogen Final Product. -----~~(b)(4)~~-----

For more details, refer to -----~~(b)(4)~~-----.

Horse tendons for production of Collagen Sponge are collected from slaughterhouses. Currently, the horse tendons are sourced from one ~~(b)(4)~~- slaughterhouse in ~~(b)(4)~~- and horse-~~(b)(4)~~- slaughterhouses in -----~~(b)(4)~~----- (horse-~~(b)(4)~~-) slaughterhouses are certified by authorities of the European Union. ----~~(b)(4)~~---- slaughterhouses possess an approval of the EU veterinary authorities and are listed on the FSIS (USDA) list as certified facilities. All slaughterhouses are regularly inspected and approved by the national veterinary authorities and are audited by Nycomed. Each batch of horse tendons supplied to Nycomed is accompanied by a veterinary certificate and by a QA certificate issued by a quality responsible person of the slaughterhouse. The certificate confirms that the horse tendons satisfy requirements for the manufacture of pharmaceutical products; that the horse tendons originate from animals free from notifiable diseases and that the meat obtained from those animals is suitable for human consumption.

VII. FINAL TACHOSIL RELEASE SPECIFICATIONS (STANDARD SIZE)
(section 3.2.P.5.1.)

| Parameter | Limits | | Analytical procedures |
|-----------------------------------|---|---|-----------------------|
| | Release | Shelf Life | |
| Appearance | Whitish sponge with one-side yellow coating | Whitish sponge with one-side yellow coating | visual |
| Length | --(b)(4)----- | - | 12237 |
| Width | --(b)(4)----- | - | 12237 |
| Adhesiveness of the coating | Abrasion -(b)(4)- ----- | Abrasion -(b)(4)- ----- | 12238 |
| Identification tests: | | | |
| Fibrinogen/Thrombin | Clot formation | - | 12254 |
| Riboflavin | Positive | - | 12241 |
| Degradability by ----(b)(4)--- | Collagen Sponge ------(b)(4)----- ----- | - | 12242 |
| Potency Assays: | | | |
| ---(b)(4)--- | ------(b)(4)----- ----- | ------(b)(4)----- ----- | 12239 |
| ---(b)(4)----- | ------(b)(4)----- | ------(b)(4)----- | |
| ---(b)(4)--- | ------(b)(4)----- ----- | ------(b)(4)----- ----- | 12240 |
| ---(b)(4)----- | ------(b)(4)----- | ------(b)(4)----- | |
| Fibrinogen/Thrombin | Clot formation within -(b)(4)- min | - | 12254 |
| Purity tests: | | | |
| ---(b)(4)----- | ---(b)(4)--- | ---(b)(4)--- | ---(b)(4)--- |
| ---(b)(4)----- ----- | ---(b)(4)--- | - | 12257 |
| Abnormal Toxicity | Requirements according to -(b)(4)- | - | ---(b)(4)-- |
| Pyrogen Test | Requirements according to -(b)(4)- | - | ---(b)(4)--- |
| Microbiological test: | | | |
| Sterility (-(b)(4)- -----) | sterile | Sterile (start and end of stability testing) | ---(b)(4)-- |

Justification of Specifications is detailed in section 3.2.P.5.6.

One (1) Page Determined to be Non-Releasable: (b)(4)

X. STABILITY (section 3.2.P.8.)

Nycomed claims the 24 month expiration period for TachoSil of all package sizes when stored at $+5 \pm 3$ °C. Original Stability data were included in the BLA and were updated on 21 December 2009 and 24 February 2010 in response to the Information Requests sent on 22 October 2009 and 25 November 2009. The study was designed according to the ICH Guideline Q1D (Bracketing and Matrixing) and includes 3 pivotal batches each for the largest size (Standard) and the smallest size (Mini) and -(b)(4)- lot for the intermediate size (Midi).

The TachoSil Final Product stability program is comprised of four major sets of data:

a) Pivotal Stability (US Validation Batches): Final Product made in November 2007 with US-sourced Fibrinogen and Thrombin in the current commercial production area (------(b)(4)----- of Bldg. -(b)(4)-). Lots ------(b)(4)----- (standard size); --(b)(4)-- (midi size) and -----(b)(4)---- (mini size) were put on stability testing. These -(b)(4)- batches were packaged in-house using identical materials to EU product.

- 18 months stability data and SAS statistics for all ongoing storage conditions (2-8°C, 25°C/------(b)(4)-----) and 6 months analytical and statistical data for storage condition at -----(b)(4)-----

b) Pivotal Stability (US Conformance Batches): Final Product made in January 2009 with US-sourced Fibrinogen and Thrombin in the current commercial production area (------(b)(4)----- of Bldg. -(b)(4)-). Lots ------(b)(4)----- (standard size), ---(b)(4)-- (midi size), and ------(b)(4)----- (mini size) were put on stability testing. These -(b)(4)- batches were packaged in-house using identical materials to EU product made on the ------(b)(4)----- and in the ---(b)(4)-- production areas.

- 9 months stability data (analytical) and SAS statistics for storage conditions at 2-8°C, 25°C/------(b)(4)----- and 6 months analytical and statistical data for storage conditions at -----(b)(4)-----

c) EU Primary Supporting Batch Stability: -(b)(4)- batches of Final Product were manufactured in September 2005 and October 2005 in the current commercial production areas (--(b)(4)-- of Bldg -(b)(4)-) with -(b)(4)-sourced Fibrinogen and Thrombin (plasma sourced in ------(b)(4)-----). These batches were packaged at a contract facility (-(b)(4)-) using identical materials to the US product and EU product made in the --(b)(4)-- production area.

- -(b)(4)- months SAS statistics for storage conditions at 2-8°C, 25°C/-(b)(4)-, ------(b)(4)----- and 6 months SAS statistics for storage condition at -----(b)(4)-----

d) EU Secondary Supporting Batch Stability: -(b)(4)- batches of Final Product were manufactured in the previous commercial production area (-(b)(4)- of Bldg -(b)(4)-) with -(b)(4)-sourced fibrinogen and thrombin (plasma sourced in ------(b)(4)-----). These batches were packaged at a contract facility (-(b)(4)-) using identical materials to the US pivotal and EU primary supporting stability batches.

- -(b)(4)- months SAS statistics for storage conditions at 2-8°C, 25°C/-(b)(4)-, ------(b)(4)----- and 6 months SAS statistics for storage condition at -----(b)(4)-----

In summary, for both the Conformance Lots and Validation Lots, all analytical stability data for the reported periods are within Specification for all storage conditions. Monitored parameters:

------(b)(4)-----

----- . There were no excursions or OOS data for any storage condition. There was no consistent trend for increase or decrease over time for Fibrinogen, Thrombin or -----(b)(4)-----.

Only slight trend for decrease was observed for Conformance Lot for stress conditions of -(b)(4)-

----- . Analytical data for Validation and Conformance Batches are summarized in the Tables below:

Summary of Analytical Data for Validation Batches

[
--(b)(4)--
]

Summary of Analytical Data for Conformance Batches

[
--(b)(4)--
]

For analysis of statistical data, I consulted with the Statistical Reviewer of the BLA. The Sponsor's statistical approach to determine the expiration date by comparing the upper and lower confidence limit to the acceptance criterion was appropriate. The statistical analysis results were reproducible. The sponsor's model to test for poolability of different batches was appropriate (the model name should be "ANCOVA" instead of "ANOVA"). The pooling of 18-month pivotal US stability data with the -(b)(4)--month primary EU supporting stability data was possible in most cases. According to ICH Guidelines Q1E, the shelf-life (based on statistical analysis) can be extrapolated 1.5 times beyond the period covered by analytical data and not more than 6 months beyond the period covered by long-term analytic data. The statistically projected expiry periods were generally longer than expiry periods extrapolated according to ICH Guidance Q1E.

For the Validation Batches, statistical analysis (based on the 18-months analytical data) reliably supports the TachoSil shelf-life of 24 months for recommended storage conditions (at 2-8°C) as

well as for 25°C/------(b)(4)----- for each package size (standard, midi and mini) and for each critical parameter - Fibrinogen, Thrombin or -----(b)(4)-----.

For the Conformance Lots of standard size, based on the statistical projections from the 9-months analytical data at $5^{\circ} \pm 3^{\circ} \text{C}$, the shortest expiry dating was -(b)(4)- months for Fibrinogen Content; -(b)(4)- months based on Thrombin Content; and -(b)(4)- months based on ----(b)(4)----. The expiry periods for other conditions are summarized in the Table below.

------(b)(4)-----

[
--(b)(4)--
]

At this time, the 9 month stability data is insufficient to reliably project the shelf-life. Nycomed committed to continue to collect stability data and perform statistical analysis of the data for the Conformance Lots, and will report the results to the Agency in annual reports.

The Primary and Secondary Supporting data reliably demonstrate stability of TachoSil for -(b)(4)- months when stored at recommended conditions.

Reviewer's comment: In conclusion, based on the analytical results and statistical projections for Validation Batches, satisfactory analytical data for Conformance Batches and statistical analysis of supporting stability data, the claimed dating period of 24 months at $+5 \pm 3^{\circ} \text{C}$ can be granted.

XI. EVALUATION OF SAFETY REGARDING ADVENTITIOUS AGENTS (3.2.A.2.)

Viral Testing of Starting Material and Viral Clearance Steps in the Manufacturing Process for TachoSil

TachoSil contains, as Active Substances, Fibrinogen and Thrombin derived from human plasma. The risk that such products will transmit viruses has been reduced by the screening of plasma donors for prior exposure to certain viruses and by testing for the presence of current specific virus infections using FDA-licensed serological assays for HBV, HIV-1/2, HCV and nucleic acid testing (NAT) assays for HCV and HIV-1/2. Furthermore, the manufacturing process for

Fibrinogen and Thrombin include pasteurization and precipitation and adsorption steps which have been validated in *in vitro* experiments and shown to be effective to inactivate and/or remove both enveloped and non-enveloped viruses (------(b)(4)-----

-----).

TachoSil contains a Collagen Sponge produced from equine tendons. The potential virus load of the tendons is considered low compared to blood or plasma. Step -(b)(4)- of the manufacturing process of the Collagen Sponge consists of a -----(b)(4)-----

----- Two studies (Study no. 2/01.105 in 2002 and Study no. 2/05.121 in 2006) were conducted (at -----(b)(4)-----
----- to validate the inactivation of potential pathogenic viruses by -(b)(4)- pH treatment at pH -(b)(4)-. The results of the validation studies indicate that -(b)(4)- pH treatment is highly effective in the inactivation of enveloped viruses (Pseudorabies virus and Parainfluenza virus, with a LRF of $\geq 6 \log_{10}$).

Finally, the product TachoSil is sterilized by gamma irradiation for --(b)(4)- sterilization. Study no. 2/02.110 was conducted in 2002 to validate the gamma-irradiation step for its virus inactivation capacity. Gamma irradiation proved effective in the inactivation of enveloped viruses (Pseudorabies virus and Parainfluenza virus, $LRF \geq 4 \log_{10}$) and non-enveloped moderately-resistant viruses relevant to the equine species (Reovirus Type 3 was used as a model virus for equine encephalosis virus, $LRF \geq 6 \log_{10}$). Gamma irradiation was moderately effective in the inactivation of non-enveloped viruses with high resistance to physico-chemical treatments (Porcine Parvovirus, LRF of approximately $3 \log_{10}$).

TABLE 3.2.A.2.4- 1: Cumulative [log10] virus reduction factors

| Virus Removal and/or Inactivation for Human Fibrinogen Active Substance | | | | | |
|--|---------------------------------|-------------|---------------------|---------------------|--------------|
| | Reduction Factors, log10 | | | | |
| Cumulative Reduction Factor (------(b)(4)----- precipitation, Pasteurization) | HIV | BVDV | HAV | CPV | |
| | ≥ 9.6 | ≥ 11.2 | ≥ 6.7 | 4.4 | |
| Virus Removal and/or inactivation for Human Thrombin Active Substance | | | | | |
| | Reduction Factors, log10 | | | | |
| Cumulative Reduction Factor (------(b)(4)----- adsorption, Pasteurization, ------(b)(4)----- ----- -----) | HIV | BVDV | HAV | CPV | HSV-1 |
| | ≥ 19.6 | ≥ 13.4 | 8.7 -(b)(4)- | 6.6 -(b)(4)- | ≥ 21.4 |
| Virus Removal and/or Inactivation for Equine Collagen Sponge | | | | | |
| | Reduction Factors, log10 | | | | |

| | | | | | |
|---|--------------|--------------|----------------------------|----------------------------|--|
| -(b)(4)- pH treatment (pH -(b)(4)-) | PRV | PI-3 | PPV | Reo | |
| | ≥ 5.7 | ≥ 5.9 | No inactivation | No inactivation | |
| Final Sterilization of TachoSil by Gamma Irradiation | | | | | |
| Reduction Factors, log10 | | | | | |
| Gamma Irradiation | PRV | PI-3 | PPV | Reo | |
| | ≥ 4.7 | ≥ 4.0 | 3.0 | ≥ 6.2 | |

The efficacy of gamma irradiation in sterilization was validated in two studies which demonstrated that the irradiation process is suitable for TachoSil and yields a sterile product with a Sterility Assurance Level of -(b)(4)- as required by -----(b)(4)---- (Please see section Validation of Sterilization Process).

Transmissible Spongiform Encephalopathy

TachoSil has an acceptable safety profile with regard to prions. TachoSil does not contain any bovine ingredients. According to current knowledge, horses do not develop prion disease. Measures to avoid cross contamination of the equine tendons are in place at the slaughterhouses. For the Active Substances - Human Fibrinogen and Human Thrombin - strict plasma donor selection criteria are employed by the manufacturer ------(b)(4)-----
-----.

XII. IMMUNOGENICITY

During review of this BLA, there was a safety concern regarding potential immunogenic responses to components of TachoSil being introduced to patients until complete degradation of TachoSil occurs. This concern was raised by the equine origin of the collagen sponge (the main excipient of TachoSil), the possibility that gamma irradiation may cause formation of neo-epitopes in active substances of TachoSil – Fibrinogen and Thrombin, and the slow absorption of TachoSil Patch. This concern was conveyed by FDA to Nycomed in the 25 November, 2010 Information Request.

The Sponsor’s responses are summarized below. The results of pre-clinical studies were discussed with the Pharmacology/Toxicology reviewer for this BLA. In addition, the immunogenicity aspect was discussed with Dr. med. vet. Lars Mecklenburg, Ph.D., Associate Director of Nonclinical Project Evaluation and Development, Nycomed Institute for Pharmacology and Preclinical Drug Safety, during Pre-License Inspection of Nycomed manufacturing facility in Linz, Austria, in December 2009.

1. The main excipient of TachoSil is a collagen patch that is produced from equine tendons. The majority of the collagen portion of tendons is made of collagen -(b)(4)-, which consists of alpha-1 and alpha-2 chains. Nycomed submitted results of comparison of the predicted primary structure of equine collagen protein and the known sequence of human collagen protein. Sequences were extracted from the NCBI protein database and their alignment was performed using the -----(b)(4)-----

----- (b)(4) ----- for alpha-1 and alpha-2 chains, respectively. High homology between human and equine proteins and high abundance of collagen - (b)(4)- molecules itself makes it unlikely that humans will raise antibodies against the equine collagen contained in TachoSil.

2. Nycomed submitted Synopses and Study Reports for animal studies investigating the risk of formation of antibodies to equine collagen. Equine collagen was non-immunogenic in guinea pigs immunized by intraperitoneal insertion of a TachoComb patch (SR 103/28) or by application of non-coated equine collagen patch (Tachotop) into skin pockets on the contralateral sides of the back (SR UL/4829). This was judged by the lack of immunological reactions (erythema, inflammation or ulceration) from 72 hours up to 140 days after implantation. No antibodies to collagen were detected by the ----- (b)(4) ---- test.

Equine collagen was immunogenic in guinea pigs only if animals were immunized with the ----- (b)(4) ----- of equine collagen and only in combination ----- (b)(4) ----- (SR UL/4830, ----- (b)(4) -----). This was evidenced by development of grade 1 erythema (SR UL/4830) and significant antibody responses evaluated by - (b)(4)- on day 60, 94 and 133 (----- (b)(4) -----). No significant antibody response was obtained in a clinically-relevant setting when a patch of equine collagen was implanted ----- (b)(4) -----.

Equine collagen in the form of a TachoComb patch was well tolerated when repeatedly administered intraperitoneally in rats (SR 103/27).

3. Gamma-irradiation used for final sterilization of TachoSil product could cause structural alterations in the fibrinogen or thrombin protein and result in formation of neoepitopes. Nycomed submitted Synopsis and Study Report for study UL/5161 in rats which investigated the potential formation of neoepitopes. Animals were immunized by subcutaneous injection of native or gamma-irradiated fibrinogen or thrombin in solution with ----- (b)(4) ----- . No evidence of neoepitope formation was revealed by ----- (b)(4) ----- . Antibody titers were determined by ----- (b)(4) ----- on days 1, 11, 29, 40, 59, 73, 85, and 106 and were found comparable for both types of -- (b)(4) --. In Reviewer's opinion, this analysis should have been supplemented by - (b)(4)- with ---- (b)(4) ---- gamma-irradiated material.

4. Nycomed submitted results of a new repeat-dose toxicity study investigating the safety of TachoSil and the antigenicity of all components of TachoSil in minipigs (SR R-BP1270). This study was not included in the original BLA and was submitted with December 21, 2009 Amendment. TachoSil was topically applied to a - (b)(4)- wound in 6 minipigs; another patch was applied to the same wound on day 2 and the third patch was applied to the - (b)(4)- on day 22 (an overall dose of 79.1 mg/kg). Three control animals received a non-coated collagen patch (Tachotop). The presence or absence of antibodies against equine collagen was evaluated pre-treatment and on days 6, 11, 26, 31 and 46 using a validated - (b)(4)- method (screening - (b)(4)- followed by confirmatory - (b)(4)- with competing antigen). Method was validated according to recent standards (----- (b)(4) -----). Positive antibody titers were detected in 2 animals of TachoSil group for equine

collagen; in 6 animals for human fibrinogen (on day 11); in 2 animals for human thrombin (on day 31) and in 6 animals for human albumin. The antibody titers were low and there was a high variability of antibody titers in pre-treatment samples. Thus immune response could not be definitely related to the use of TachoSil.

None of the minipigs revealed any evidence of systemic effects of antibodies leading to impaired hemostasis (assessed by thromboplastin time and partial thromboplastin time). This suggests that detected antibodies are not of clinical relevance for the organism. TachoSil was effective upon application to the (b)(4)- wound (after two applications to the (b)(4)- wound).

5. Development of antibodies against equine (b)(4)- collagen or against neoepitopes in fibrinogen or thrombin in humans pose a safety concern if these antibodies cross-react with endogenous counterparts. According to literature, autoantibodies against collagen (b)(4)- are associated with systemic sclerosis (scleroderma) in humans (Mackel et al., 1982; Riente et al., 1995). Lesions resembling systemic sclerosis have not been reported from clinical studies with TachoSil (----- (b)(4) -----). The toxicity studies using either TachoSil or one of its predecessors did not demonstrate any signs of autoimmunity against collagen (Toxicology Written Summary, Section 2.6.6.9). Neutralizing antibodies to fibrinogen or thrombin would result in impaired general hemostasis. The clinical experience with TachoSil has not revealed any such concerns (----- (b)(4) -----).
6. The concern that antibody formation could reduce the hemostatic efficacy of TachoSil is rather theoretical considering that formation of fibrin clot occurs within 3-5 minutes upon patch application.
7. Nycomed is currently validating (b)(4)- assays (according to ICH guidelines Q2A and Q2B) based on the ----- (b)(4) ----- for detection of antibodies to fibrinogen, thrombin, albumin and equine collagen, with confirmation of specificity by inclusion of a competing antigen.
8. Nycomed plans to investigate the potential formation of antibodies against components of TachoSil in ----- (b)(4) ----- which will be performed under IND (b)(4)- currently submitted to FDA. This is an open, randomized, controlled, parallel-group, multicenter trial comparing TachoSil with ----- (b)(4) -----
-----.

Reviewer's comments:

The results from pre-clinical studies indicate that TachoSil does not appear to be immunogenic. The safety profile determined for TachoSil is satisfactory to allay the immunogenicity concern to the extent which studies in animals can support (see Guidance ICH M3 for Pharmacological/Toxicological studies). Further evaluation of immunogenicity of TachoSil will be performed in clinical studies conducted by Nycomed under an Investigational New Drug application (IND) ----- (b)(4) ----- the indications for TachoSil. Therefore, no PMR/PMC to evaluate immunogenicity is required to support the approval of this BLA.

XIII. MANUFACTURING FACILITY

The review of the manufacturing facilities is responsibility of the DMPQ reviewer.

The Center for Biologics Evaluation and Research (CBER) performed a pre-license inspection for TachoSil (STN 125351/0 at Nycomed Austria GmbH facility in Linz, Austria, from December 10th to December 17th, 2009. This was the first FDA inspection of the TachoSil production area which was constructed in 2004 on the -----(b)(4)----- of Nycomed's Building -(b)(4)-. The inspection included the complete manufacturing process for TachoSil which occurs on the -----(b)(4)----- of Building -(b)(4)-.

No 483 was issued at the end of this pre-license inspection for TachoSil.

RECOMMENDATION

The manufacturing process is validated and ensures the reproducible manufacture of TachoSil at production scale. The analysis of purity, identity and coagulation properties of the components of TachoSil, its clot formation characteristics and physiological sealing/gluing properties supports comparability of TachoSil product intended for the US market and the licensed TachoSil product currently on the European market. The manufacturing processes for Active Substances at -(b)(4)- and Collagen Sponge at Nycomed as well as the sterilization of TachoSil Drug Product by gamma irradiation provide acceptable safety margins regarding adventitious agents. The Product reviewer recommends **the approval** of this BLA.