Com/92/2/54

**PL Number:**

0116/0233-5

**Company:**

Baxter Healthcare Ltd

**Product:**

Recombinate
250, 500 and 1000IU

**Therapeutic Classification:**

Recombinant Clotting Factor

**Active Constituent:**

Factor VIII

**SUB-COMMITTEE ON SAFETY, EFFICACY AND ADVERSE REACTIONS**

**Recommendations**

On consideration of the evidence before them the Committee advised that a product licence should be granted for this preparation on condition that satisfactory responses to the following comments are provided to the CPMP.

1. Liver function data for the study in previously untreated patients should be provided.

2. The summary of product characteristics should be amended in particular:

**2.1** The statement that preclinical studies have shown that Recombinate is safe and effective should be deleted, from the sections on Undesirable Effects and Pharmacological Properties.

**2.2** The statement in the indications section, that Recombinate may be considered as a primary treatment option in patients not previously exposed to human blood derivatives should be deleted.

Date: July 1992 CSM/Biologicals/92/3 Meeting Appendix

SUBCOMMITTEE ON BIOLOGICALS

RECOMMENDATIONS

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(CPMP)

Company
Baxter Healthcare
Ltd

Product
Recombinate
250IU, 500IU
1000IU

Therapeutic Class
Clotting Factor

Active
Constituent
Antihaemophilic
Factor
(Recombinant)
[Company Term]

On the evidence before them, the subcommittee recommended that the following comments and points should be forwarded to the CPMP/Rapporteur:

i) Dutch rapporteur questions

The UK supports the pharmaceutical questions raised by the Dutch Rapporteur. These questions are appended.

ii) Additional UK pharmaceutical questions:

Part I

IA ADMINISTRATIVE DETAILS

1. It would be helpful if in section 1A3(d) the manufacturers of the monoclonal antibody and immunoaffinity resin could be indicated. (remark)

IB SUMMARY OF PRODUCT CHARACTERISTICS

2. In the SPC, product literature, labels etc a suitable name should be used for the drug substance which distinguishes it from native Factor VIII and other recombinant Factor VIIIs. A suitable approved name for the drug substance should be applied for. (Major Point)

3. It would be helpful if the average specific activity for the drug substance was given in the SPC. (point for clarification)

PART IIA

DEVELOPMENT PHARMACEUTICS

4. The acceptable ranges of excipients in the finished product appear to be wide, eg a two fold range of albumin. [This is wider than for another monoclonal antibody purified FVIII, Hemofil-M, produced by the same Company]. The rationale for the ranges of excipients should be given (point for clarification)

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PART IIB

MANUFACTURE OF THE DOSAGE FORM

Company
Baxter Healthcare Ltd

4. The bioburden of the pre-sterile filtration solution should be monitored. (point for clarification)

Product
Recombinate
250IU, 500IU
1000IU

Part IIC

DRUG SUBSTANCE SPECIFICATIONS

Therapeutic Class
Clotting Factor

5. The Company note that they have been unable to fully characterise the drug substance. The rationale for not including a range of physico-chemical tests (in addition to SDS-PAGE) into the drug substance specifications should be given and justfied. (major point)

Active Constituent
Antihaemophilic Factor
(Recombinant)
[Company Term]

6. Unless justified, the carbohydrate composition should be monitored. (major point)

7. The Company have given only a minimum potency in the drug substance specifications. The drug substance is a recombinant molecule with only low amounts of impurities. Therefore it would also be of concern if the drug substance was to become significantly more potent than that established. Suitable upper and lower limits for mean result and fiducial limits should be set. (point for clarification)

8. The specifications should include a test for activity using a suitable chromogenic method. (point for clarification)

9. The result of the bioassay is given in units while the expression of strength of the dosage form is given in IU. the position should be resolved. (point for clarification).

GENETICS

10. In volume 2 p286 (IIC) the vWF is stated to contain a polyoma enhancer element. It should be clarified whether the construct contains polyoma or SV40 sequences. (point for clarification).

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[Company Term]

11. In volume 7 pp538-40, batch 9A03H055B consistently gives restriction maps shifted from other samples run on the same gels. The Company should be asked to comment on this. (point for clarification)

12. It should be indicated whether the genes for rAHF and rvWF are located on the same chromosome. It should be indicated on which chromosome the genes are located. It should be indicated whether there is any loss of stability (eg in yield) because the chromosome(s) or genes for rAHF or rvWF are lost at different rates during cell culture. (point for clarification)

13. The host-vector system should be monitored at the end of cell culture. (major point)

CELL BANKS

14. It should be clarified whether both WCB K and P are used currently. The size and rate of use of cell banks should be clarified. It should be clarified whether there are any differences in the population doubling levels between the two cell banks K and P. (point for clarification)

CELL CULTURE

15. It is indicated (eg IIC p370) that back inoculation of bioreactors can occur. It should be indicated under what circumstances this can occur. It should also be indicated whether product produced from such bioreactors will be from cells exceeding the population doubling level at which stability has been demonstrated. It should be indicated whether back inoculation can occur more than once. (point for clarification)

16. During cell culture the 2500L bioreactors are harvested about every three days until 65 population doublings. The usual duration of the cell culture process and the number of harvests should be indicated. (point for clarification)

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[Company Term]

RECOMMENDATIONS

17. The circumstances under which it is necessary to to concentrate cells before adding fresh medium during cell expansion should be clarified (point for clarification)

18. It is indicated that each 2500L bioreactor is harvested every three days, and that filtered conditioned media from several bioreactors is loaded onto the first chromatography resin as a single batch. Details on the storage of harvests should be given together with any limits on the length of storage of harvests or any affect of storage on stability. (point for clarification)

19. It should be indicated if there are any differences in the rAHF produced at different times during the cell culture process (eg yield, physico-chemical characteristics such as Western Blot). (major point)

PURIFICATION

20. It should be confirmed that reprocessing in drug substance manufacture will not occur. (point for clarification)

21. The ranges for in-process controls applied to recovery from the three chromatographic steps (eg Pharm Exp Rep, IIC:1.2.4) may be wide in the light of production experience and consideration could be given to tightening them. It is unclear how the upper limit of 125% recovery for the Mono S step can be achieved. (point for clarification)

MONOCLONAL ANTIBODY/COLUMN

22. The Company (Baxter) also produces another Factor VIII preparation (Hemofil-M) which is purified using a monoclonal antibody column. Hemofil-M is derived from human plasma. It appears that the same murine hybridoma (F8.1.5.6) is referred to for Hemofil-M and Recombinate. Therefore:

22.1 It should be clarified whether the monoclonal antibody used to make the affinity column for use in the manufacture of Recombinate and Hemofil-M are in fact exactly the same. (point for clarification)

22.2 It should be confirmed that the manufacturing process for Recombinate uses dedicated columns. (Major Point)

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[Company Term]

23. It is indicated (p464) that the donors for human transferrin used in MAb cell culture are tested for HTLV III and HBsAg. It should be indicated if testing includes antibodies for HIV 1 and 2, and HCV. The human transferrin should comply with the CPMP guidelines 'Medicinal products derived from human blood and plasma'. (point for clarification)

24. The Company (Baxter) also produces another FVIII product (Hemofil-M, plasma derived) which appears to be purified using the same MAb. It is indicated in the Hemofil-M file that cattle used as source for bovine materials used in MAb manufacture, may be fed ruminant feed. The position for Recombinate should be clarified and if necessary justified. It should be indicated whether CPMP guidelines on BSE are complied with. (point for clarification)

25. The viral inactivation/removal studies to be performed on the Hyland procedure for MAb purification should take account of the CPMP guideline on viral validation. [Supplements question 5 from the rapporteur]. (Remark)

26. It is noted by the Company (eg IIC p419-20) that some instability of the hybridoma is observed. The Company should ensure that this is not compromising their ability to produce consistent batches of antibody with the characteristics necessary for production of consistent FVIII products. The Company should provide reassurance that the instability is not more of a problem with the production procedure used by Highland Hayward compared with Celltech. (point for clarification)

27. Brief details of the bioreactors used for monoclonal antibody production should be given. (point for clarification)

28. Details of the method for the rAHF inhibition assay referred to in the MAb acceptance criteria (GI) on p504, and the Factor VIII function test cross referred to on p499 should be given (point for clarification)

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29. The purification of the MAb is described vaguely; eg pp492-3, the columns used for purification of the MAb are described only as 'anion exchange chromatography' or 'mixed mode ion exchange'. The columns used should be specified. (point for clarification)

30. The Company note that it is difficult to fully characterise the drug substance, rAHF. Therefore control of the method of manufacture is important to ensure consistency of the drug substance. The monoclonal antibody immunoaffinity column is a key step in the purification process. As two manufacturers of the MAb are proposed, the acceptance criteria for physico-chemical tests should be improved (rather than 'report result' or '>95%' etc) so that MAb from either site of manufacture is comparable. (This supplements the question from the Rapporteur asking for the results of a study of a direct comparison of material from the two sites). (major point)

31. At the moment the description of the manufacture of the immunoaffinity matrix is brief (reference is made on p503 to a flow diagram). The manufacture should be described. (major point)

32. If a different vendor is used for immunoaffinity matrix production (as mentioned on p452), the Marketing Authorisation should be varied. (remark)

33. The specifications for the F8.1 monoclonal antibody Sepharose CL-4B immunoaffinity resin should be improved to include a test (with suitable limits) to ensure that factor VIII does bind sufficiently to the column. (major point)

von WILLEBRAND FACTOR

34. The Company's production strategy uses genetically engineered CHO cells which coexpress rvWF (in order to stabilize the secreted rAHF). However, the structure of their rvWF has not been clearly given. It is not indicated whether the rvWF secreted from the cells has the amino acid sequence predicted from the gene sequence utilised or whether post translational modification occurs. It is not clear whether there are differences (eg amino acid sequence, glycosylation etc) between the rvWF produced and 'native' vWF. The position should be resolved (major point).

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IMPURITIES

35. It should be clarified whether any rAHF related proteins (eg aggregates etc) or whether non rAHF proteins are seen on SDS-PAGE. If necessary appropriate tests with suitable limits should be introduced into specifications. (point for clarification)

36. The calculated clearance factors (p562) for some impurities does not appear to support the proposed limits eg p774. For example, the proposed limit for CHO proteins and BSA appears high (limits proposed 1ug and 0.8ug per 1000IU, assuming minimum specific activity of 4000IU/mg this is equivalent to about 0.4% and 0.32%). The position should be clarified. (major point)

BATCH ANALYSES (DRUG SUBSTANCE)

37. The batch analyses for the drug substance given on pages 807-11 give the results of some tests as "meets specification" which makes it difficult to assess the consistency of the product. (point for clarification).

38. The confidence intervals for bioassay results should be given. (point for clarification)

39. In the Pharm Exp Rep (IIC 1.2.8) it is indicated that batch analyses for material produced in 1989 are provided. Data from later batches should be provided, if available. (point for clarification)

Part IIE

DOSAGE FORM SPECIFICATIONS

40. The acceptance criteria for the immunoblot test is given in the specifications (IIE p826-7) as 'complies to specification'. The acceptance criteria should should be improved and should unless justified specify which bands may be present. (major point).

41. Baxter also produce another FVIII (plasma derived), Hemofil-M. It appears that the bioassay proposed for Hemofil-M and Recombinate are different (two stage and one stage) assays even though both products are monoclonal antibody purified FVIIIs produced by the same Company. The position should be clarified and justified. (point for clarification)

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[Company Term]

BATCH ANALYSES OF FINISHED PRODUCT

42. It was unclear from the batch analyses given on p838-9 what the nominal potency of the batches were. It is therefore difficult to assess whether the bioassay results were supportive of the proposed specification limits. Confidence limits for bioassay results were also not given. The position should be clarified. (point for clarification)

IIF

STABILITY OF THE DRUG SUBSTANCE

43. It should be indicated whether aggregates or degradation products have been seen on storage. It is unclear whether the acceptance criteria for SDS-PAGE or RP-HPLC tests would exclude bands or peaks not normally seen. As the results of stability studies (eg p851) are given as 'meets specification' or 'conforms' it is difficult to assess whether any degradation is seen. (point for clarification)

STABILITY OF THE DOSAGE FORM

44. The data supplied so far does not clearly support a shelf life of 2 years at 25 C. The potency (appendix IIF:2.3 a-3) appeared to fall when stored at higher temperatures eg to below 90% at 25 C or 30 C. However, when stored at 5 C no real trend for loss of activity was seen. This infers that degradation at 25 C is significantly higher than at 5 C. This may be particularly important as the Company have noted that it is difficult to fully characterise the drug substance. The proposed shelf life should be reduced or justified. (major point)

45. There is a suggestion of an increase of activity on storage of some batches. This could indicate activation since only one-stage assays are used. Additional assays by two-stage or chromogenic methods should be used to check this. (point for clarification)

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46. Unless justified, it should be indicated whether aggregates or degradation products of rAHF are seen on storage at the proposed maximum temperature of storage. (major point)

47. The results of the immunoblot test in stability studies on the dosage form (appendix IIF:2.3 a-3 p14) are given as 'pass'. It should be indicated if any bands not normally seen are observed. (point for clarification)

PART IIQ

48. It was unclear how anti-FVIII antibody in patients had been measured: by inhibition of FVIII assay or by direct measurement of anti-FVIII antibody. The position should be clarified. (point for clarification)

49. It should be confirmed that all sterile device components provided with the product comply with EEC requirements for sterile devices. Brief information on these devices should be provided, eg supplier, materials of which they are composed, methods of sterilisation and packaging etc. (point for clarification)

Part V

50. Appropriate samples of Recombinate should be provided. (remark)

51. In view of the complex nature of the drug substance, Recombinate should be subject to product monitoring. (remark)

RAPPORTEUR (DUTCH) PHARMACEUTICAL QUESTIONS APPENDED TO BIOLOGICALS SUBCOMMITTEE DRAFT RECOMMENDATIONS (JULY 1992)

**Recombinate, poeder voor injectievloeistof RVG 16030/-1/-2**
Assessment Report Bilthoven 12-5-1992
LGM 540/92 PJ

CONCLUSION

The following objections are present:

Part II C 1: Specifications and routine tests

1. Potency assay: A requirement for the fiducial limit of error of the estimated potency is lacking and should be added to the specification (fiducial limits of error (p=0.95) of the estimated potency relative to the stated potency).

Part II C 1: Genetic stability

2. The stability of the cell culture during a common production run has been validated at the RNA and DNA level. For recombinant proteins it is common to demonstrate sequence-stability at both the DNA and the protein level.
The Expert Report states (page 36) "the product produces at 127 generations was indistinguishable from that produced at < 65 generations". Supporting data (analysis methods, results) of this statement could not be found in the dossier and -in order to support the stability of the produced protein sequence- are asked for.

Part II C 1: Purification process

3. The reuse criteria for chromatographic columns should be stated more precisely.
Limits for the duration of use and/or number of runs should be considered once more experienced has been gained.

Part II C 1: Monoclonal antibodies used in immunoaffinity chromatography

4. Monoclonal antibody batch analysis results of 3 Celltech batches and 5 Hyland batches are submitted. However, a conclusive comparison of these results is not possible due to differences in in-house analysis (Celltech vs. Hyland) methods and (presumably) reference preparations.
In order to ensure biological equivalence of the monoclonal antibodies produced by both processes comparative results of batches produced by both processes should be provided, e.g. direct comparative analysis of batches from both processes on the same SDS-PAGE and (narrow pH-gradient) IEF gels (submit clear photographs) and quantitative results of r-AHF binding studies.

5. The in-process testing of the Hyland F8.1 production process should be described in more detail. It is unclear which in-process tests on microbial and viral contamination are performed during each production process. Moreover the capacity of the Hyland F8.1 purification process to remove/inactivate potential viral impurities should be adressed.

Part II C 2: Human albumin solution

6. In addition to the quality reference 'Ph.Eur. monograph' it should be stated more explicitly which virus tests will be carried out on blood donations. In this aspect the albumin ought to comply with the CPMP Note For Guidance 'Medicinal products derived from human blood and plasma' (coming into operation 1 may 1992).

**Recombinate, poeder voor injectievloeistof RVG 16030/-1/-2**
Assessment Report Bilthoven 12-5-1992
LGM 540/92 PJ

Part II E

7. Potency specification: The requirement for the fiducial limits of error of the assay should be added to this specification.

8. The application of an one-stage assay in the potency determination of Recombinate should be justified by means of cross validation data (several batches) against the Ph.Eur. two-stage assay (using the MEGA 1 standard).

Csm/92/7/5A

COMMERCIAL IN CONFIDENCE

| | |
|---|---|
| TYPE OF APPLICATION: NAS | NUMBER: PL0116/0233-5 |
| PROPOSED CERTIFICATE/LICENCE HOLDER: BAXTER HEALTHCARE LIMITED | PRODUCT NAME: RECOMBINATE |
| MANUFACTURER OF DOSAGE FORM: HYLAND/BAXTER LOS ANGELES | THERAPEUTIC CLASSIFICATION: CLOTTING FACTOR |
| LEGAL STATUS: POM / SALE/SUPPLY: HOSPITAL/CLINICS | RECEIVED: 1 MAY 1992 |
| | MEETING : JULY 1992 |
| First recombinant Factor VIII | COMMITTEE ON SAFETY OF MEDICINES |
| | SUB-COMMITTEE: SEAR |
| | CONSIDERATION BY OTHER SUB-COMMITTEES: BIOLS |
| | ASSESSED BY: DR ROTBLAT<br>DR LEE<br>DR GATE |

INDEX:

## INTRODUCTION

This is an application for a Marketing Authorisation for Recombinate. The active ingredient is a recombinant factor VIII, and the active ingredient is referred to by the Company as antihaemophilic factor (recombinant), rAHF. The active ingredient is produced in a genetically engineered Chinese Hampstead ovary cell line. The purification process involves the use of a monoclonal antibody affinity column.

It is a CPMP concertation application (No 43) and the Netherlands is acting as Rapporteur.

Recombinate injection is presented as a lyophilised powder and is for intravenous administration following reconstitution with the accompanying diluent, water for injections. Three strengths are proposed, nominally 250, 500, or 1000IU per bottle.

The proposed indication is in acquired and congenital factor VIII deficiency for the prevention and control of haemorrhagic episodes.

## STRUCTURE OF rAHF

The precise structure of rAHF has not been defined precisely by the Company. The Company note that the primary translation product is subject to post-translational modifications which result in a series of variants.
Given below is a narrative description of the structure of rAHF, and given on the next pages are flow diagrams indicating the post-translational modifications.
The primary translation product of rAHF mRNA is a 2351 amino acid protein. During transit through the lumen of the endoplasmic reticulum, a 19 amino acid leader peptide is cleaved from the amino terminus. The resulting 2332 amino acid precursor protein is further cleaved proteolytically to generate amino terminus 'heavy chain' peptides that range in size from 90kD to approximately 200kD, and carboxy terminus derived 'light chain' peptides of approximately 80 kD ( and approximately 120 kD for a minor variant. Although rAHF heavy chains are heterogeneous in length, they have a uniform amino terminal sequence and are extensively glycosylated with both N-linked and O-linked carbohydrate. The light chains of rAHF are also glycosylated, although not as extensively as the heavy chains. Amino terminal sequence analysis of the light chain indicates that two species predominate: one begining at amino acid 1649 and another begining at amino acid 1658. In addition, a relatively small fraction of light chain in the rAHF complex is in the form of a 120 kD peptide that begins at amino acid 1314. The heavy and light chain subunits of rAHF remain associated, stabilised as a metal (Ca++) complex that can be disrupted by the addition of chelating agents such as EDTA.

Added by UK Secretariat.

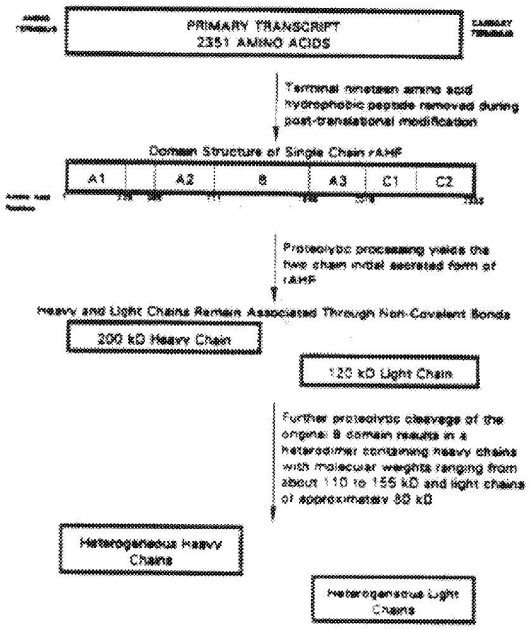
| Name of Company: **Baxter Healthcare Corporation Hyland Division** | Summary Table Referring to Part IIC:1.2.3 of the Dossier | (For National Authority Use Only) |
|---|---|---|
| Name of Finished Medicinal Product: **Recombinate™ Antihemophilic Factor** | | |
| Name of Ingredient: **Antihemophilic Factor (recombinant)** | | |

PART IIC: CONTROL OF STARING MATERIALS. NON-PHARMACOPOEIAL ACTIVE INGREDIENTS: SCIENTIFIC DATA (DEVELOPMENT CHEMISTRY) (VOLUME 2)

| Evidence of molecular structure: Part IIC:1.2.3 b Pages 274-325 | (For National Authority Use Only) COMMENTS: |
|---|---|

**Figure IIC-6**

Post Translational Proteolytic Processing of Antihemophilic Factor






2A

Added by UK Secretariat

## FIGURE IIC:1.2.2 b-1

### rAHF Structure

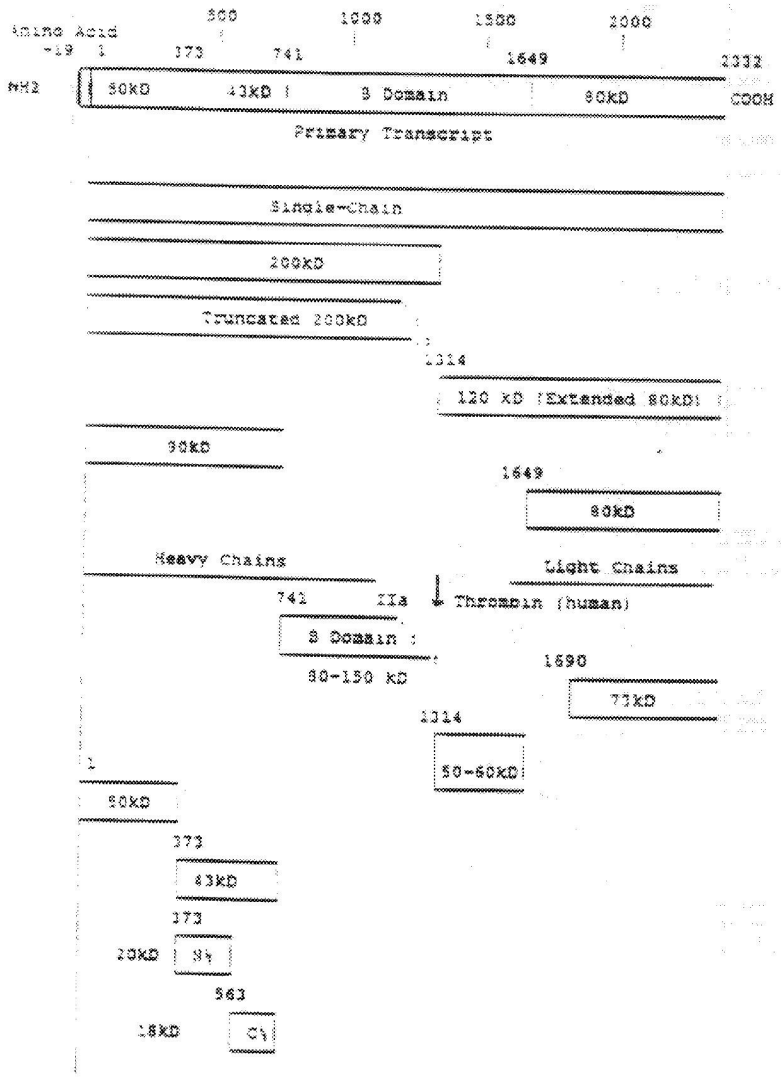



*Figure IIC:1.2.2 b-1.* During secretion, the primary translation product is processed into two subunits: the amino-terminal heavy chain and carboxy-terminal light chain. Thrombin (IIa) digestion activates and then inactivates rAHF.

~~PAGE 256~~



2B

PHARMACEUTICAL SUMMARY OF THE DEVELOPMENT AND MANUFACTURE OF RECOMBINATE

INTRODUCTION

Presented here is a summary of the pharmaceutical aspects of the development, production and manufacture of Recombinate. A more detailed account is presented by the Company Pharmaceutical Expert and his report is part of the Rapporteur's (Netherlands) assessment report and is presented in the Biologicals subcommittee paper (July 1992).

Recombinate injection is presented as a lyophilised powder and is for intravenous administration following reconstitution with the accompanying diluent, water for injections. Three strengths are proposed, nominally 250, 500, or 1000IU per bottle.

The active ingredient is a recombinant factor VIII, and the active ingredient is referred to by the Company as antihaemophilic factor (recombinant), rAHF. The drug substance is produced in genetically engineered Chinese Hampstead ovary (CHO) cells and part of the purification process involves the use of a monoclonal antibody affinity column.

The development and production of Recombinate has been divided into segments for ease of description: drug substance (including genetics), dosage form, and monoclonal antibody column.

DRUG SUBSTANCE

For the structure of the drug substance please see the section 'structure of rAHF' above.

The drug substance is produced in genetically engineered CHO cells. Historically two cell lines have been used. The VIII-H9 cell line was employed in a pilot production run to make material for phase I clinical trials early in the development of rAHF. The 10A1C6 line was employed for all subsequent production and scale up to make material for clinical trial and commercial use. The Company note that there have been four clinical production campaigns:

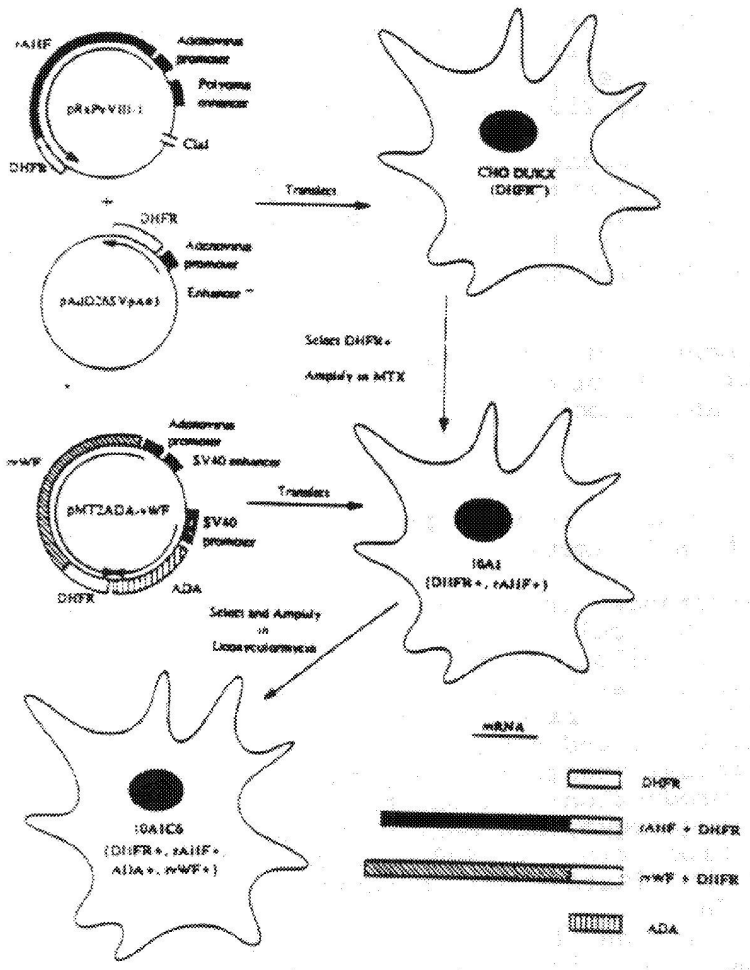
- 1986 production (~400L harvest scale) at contract facility, Summa, Albuquerque, New Mexico, using VIII-H9 cell line.
- 1988 production (~1400L harvest scale, 1600L bioreactor) at Genetics Institute (GI) Cambridge USA facility, using cell line 10A1C6 (WCB K).
- 1988 campaign (~2100L harvest scale, 2500L bioreactor) at GI Andover manufacturing facility, using cell line 10A1C6 (WCB P).
- 1989 onset of commercial scale production (~6000L harvest scale, three 2500L bioreactors) at GI Andover facility using cell line 10A1C6 (WCB P).

The Company have provided little information on cell line VIII-H9. However, it appears that the expression vector used for integration contains the full length AHF cDNA sequence and expresses DHFR. The VIII-H9 was used in serum containing medium in roller bottle production.

2C

## FIGURE IIC:1.2.3 b:1.3-1

Derivation of rAHF and rAHF-rvWF Coexpressing CHO Cell Lines



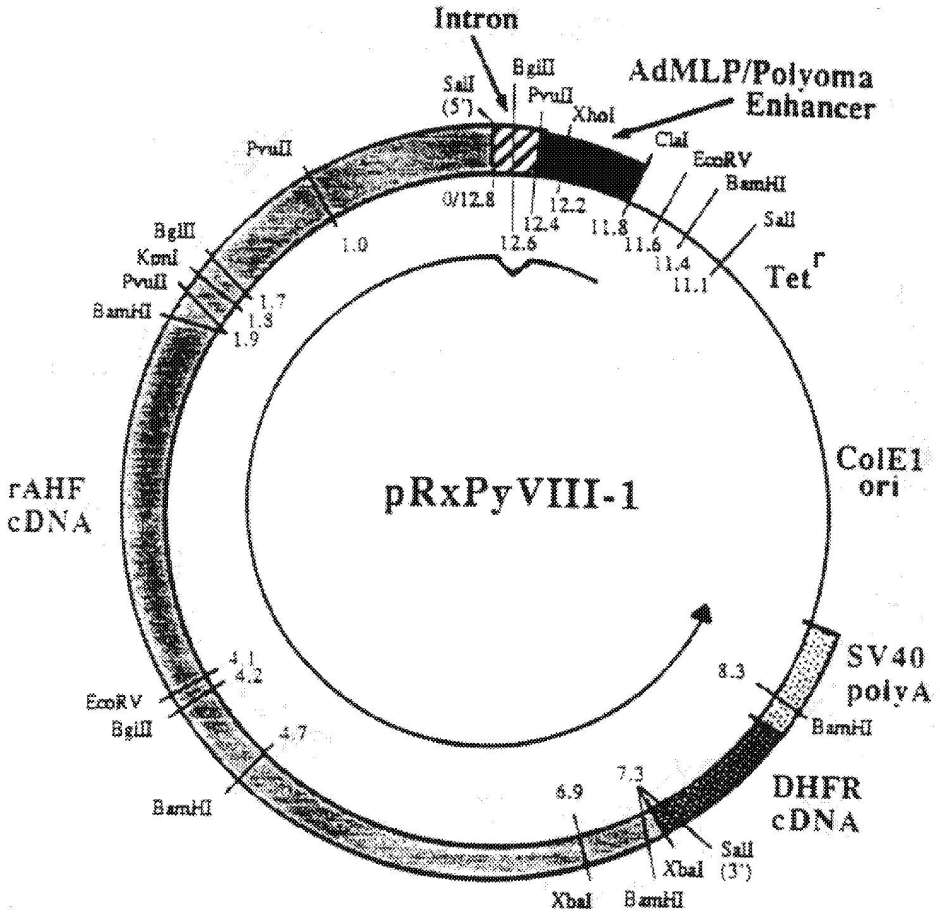





2 b

## FIGURE IIC:1.2.3 b:1.1.2-1

rAHF Expression Vector pRxPyVIII-1




A legend ~~to Figure IIC:1.2.3 b:1.1.2-1 appears on the following pages~~.

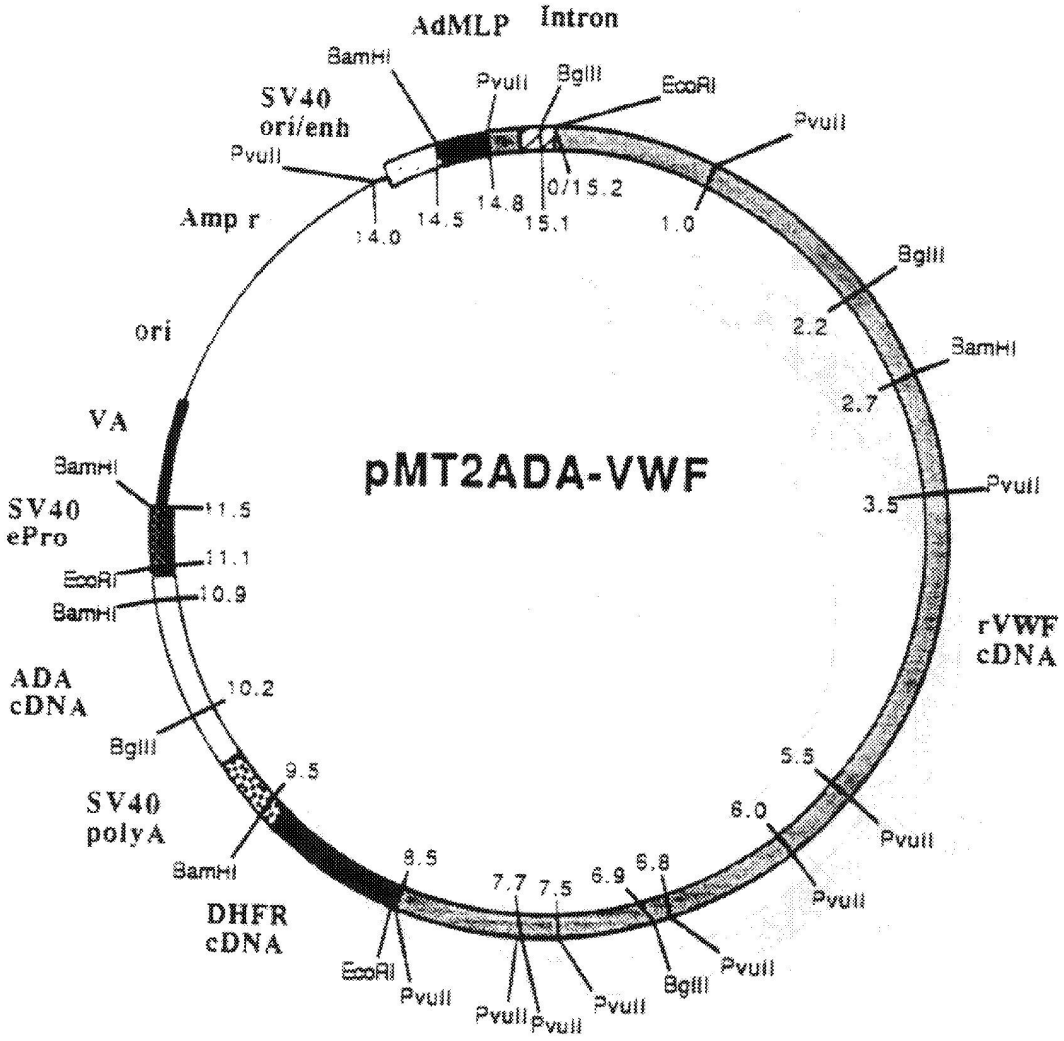




2 ∈

FIGURE IIC:1.2.3 b:1.1.2-2

rvWF Expression Vector pMT2ADA-vWF




~~A legend to Figure IIC:1.2.3 b:1.1.2-2 appears on the following pages.~~





2F

Human genomic DNA sequences encoding factor VIII amino acid sequences were used as probes to detect cDNA clones from a human foetal liver cDNA library. Four overlapping cDNA clones were combined to give rise to a single full length cDNA sequence which was used for the cell lines. Generation of cell line 10A1C6 (see diagrams on previous pages) required the co-integration of two vectors, one coding for rAHF and one for DHFR expression for methotrexate selection, followed by integration and amplification of a third vector for recombinant von Willebrand factor (rvWF) and adenosine deaminase with dCF (2'-deoxycoformycin) selection. The apparent amplification unit for the rAHF contains two copies of the gene, one complete and intact, the other scrambled and partially deleted. The Company indicates that transcripts corresponding to the truncated gene in practice have not been detected, and the Rapporteur notes that the Company have provided sufficient evidence that the truncated AHF gene will not be expressed. The gene copy number for rAHF and rvWF is about 500 and 20 respectively. The coexpressed rvWF stabilizes the rAHF produced by the cells.

After adaptation to suspension culture and serum free growth, two WCBs have been constructed. 10A1C6 WCB K and 10A1C6 WCB P. These two WCBs differ only in that MCB/WCB K were established by adaptation of 10A1C6 cells to serum free culture conditions prior to removal of selective pressure (2'deoxycoformycin), whereas the MCB/WCB P were established by adaptation to serum free culture conditions after removal of selective pressure. The cell line 10A1C6 is characterised for freedom from microbial contamination at the WCB level and after extended culture.

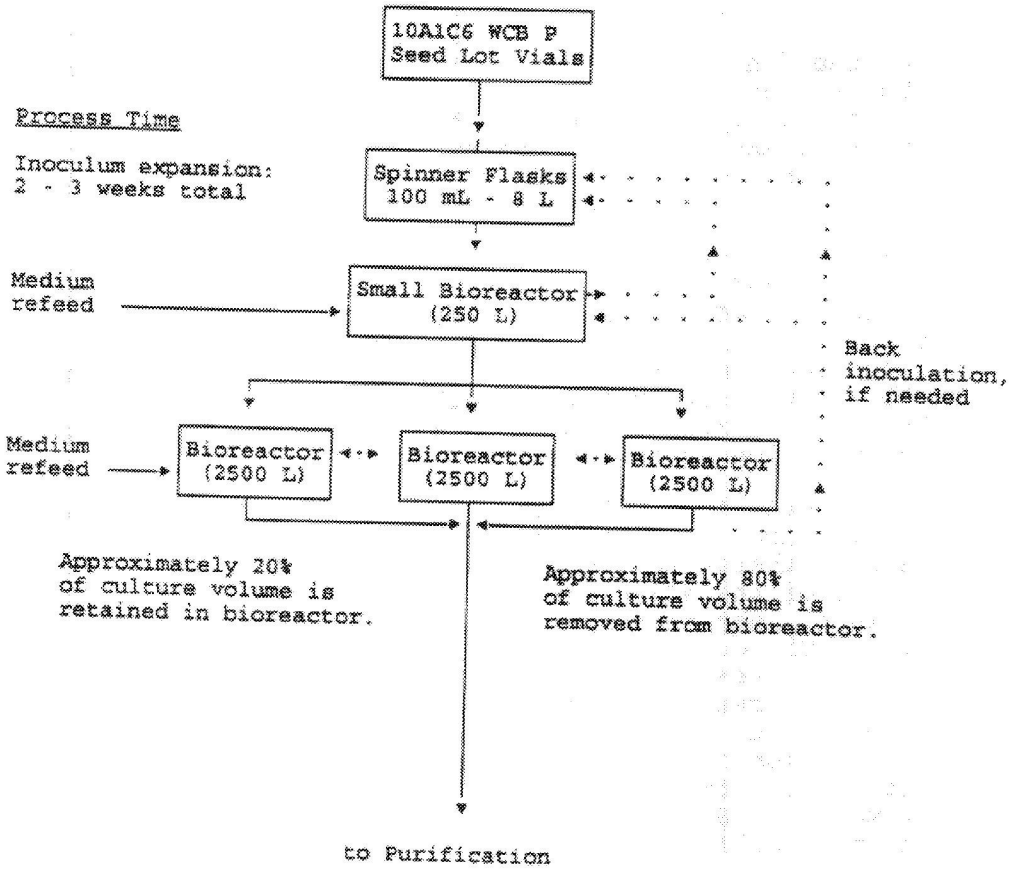
Two media are used for cell culture. Medium 072, which contains methotrexate, is used for the first 8 - 12 generations from the WCB. Medium 054 is used thereafter. Both media contain BSA.
Vials (2 to 8) of the WCB are expanded in spinner flasks. The culture is expanded into increasingly larger spinner flasks using a batch refeed process. The cell culture process is continued, in batch refeed mode, in a 250L bioreactor, which is then used as inoculum for a 2500L bioreactor also in batch refeed. Three 2500L bioreactors are used. The source of the inoculum can be another 2500L bioreactor. After about three days a portion of the medium is removed (typically 2000L), which is then refed with fresh medium. The batch refeed process continues until a maximum of 65 generations. Validation studies have included studies on extended cell cultures.

Cells are separated from the conditioned medium by filtration. The filtered conditioned medium is then processed on a monoclonal antibody column (2 x 15L). Filtered conditioned media from several bioreactors are loaded onto the resin as a single batch. After isocratic elution from the immunoaffinity system, the eluate is loaded onto a cation exchange (Mono S) chromatography system (1.7L). This column is washed then eluted with with an increasing salt gradient. The selected Mono S eluate fractions are pooled and loaded onto the Mono Q chromatography system (320ml) in the final step of the process. This column is eluted in an increasing salt gradient. The selected Mono Q eluate fractions are pooled and stored at -80 C. These pooled fractions comprise bulk Antihemophilic Factor (recombinant).

2 G

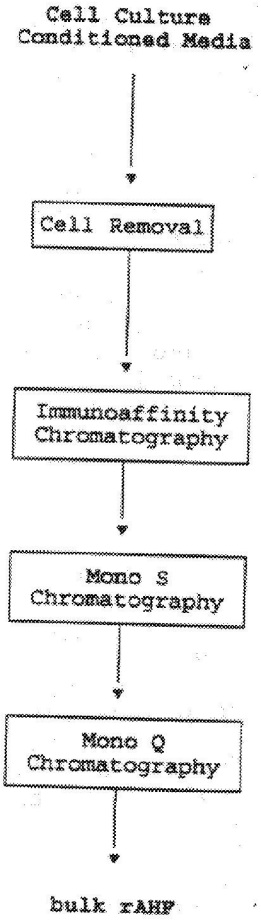
## FIGURE IIC:1.2.3 d:1-1

### Flow Diagram of Cell Culture Process



## FIGURE IIC:1.2.3 d:2.1-1

### Flow Diagram of Purification Process



Upon completion of production of the drug substance, the bulk rAHF is transferred on dry ice from GI, Andover to Baxter Healthcare Corporation, Hyland Division, Los Angeles.

## DOSAGE FORM

The finished product is formulated as a sterile lyophylised powder for iv injection following reconstitution with WFI. Three strengths are proposed, 250, 500 and 1000IU. The excipients present are albumin, sodium chloride, histidine, polyethylene glycol, and calcium chloride. Recombinate is presented in a 30ml clear glass bottle with rubber stopper and protective aluminium caps.

For the manufacturing of Recombinate, one to six batches of bulk rAHF may be used to prepare finished product depending on the strength of the finished product. The 'pooled' bulk rAHF is assayed. The pooled bulk is diluted 1:3 with buffer solution and assayed again. The solution is further diluted with another buffer to a 'target' and assayed again. This potency is used to dilute the solution to the desired value for the dosage form. The potency is rechecked. After adjustment of the pH, the solution is sterilised by filtration (0.22u). The sterile solution is dispensed into the glass bottles and then freeze dried. The bottles are stoppered under vacuum and capped. Released vials can be labelled at the US site or in the facility in Lessines (Belgium).

The proposed shelf life of the lyophylised product is 24 months at 2 - 25 C. After reconstitution it is recommended that it is used within 3 hours.

## MONOCLONAL ANTIBODY (MAb) IMMUNOAFFINITY COLUMN

GI prepared and characterised the F8.1.5.6 hybridoma cell line which produces the F8.1 monoclonal antibody used in rAHF purification. The hybridoma was obtained by fusion of a non-secreting line with spleen cells obtained from mice immunised with plasma derived factor VIII. The F8.1 MAb is to the 43kD thrombin fragment on the AHF heavy chain. Aliquots of the cell line were transferred to Celltech (UK) and to the Hyland Hayward facility for large scale production of the monoclonal antibody. Each Company established WCBs.

Each Company uses a different method of cell culture and purification for the MAb. Celltech uses essentially a batch process, and purification involves protein-A, IE chromatography (DEAE Sepharose FF), diafiltration and sterile filtration. The process at Hyland uses a continuous process and purification involves Fast Flow Sepharose chromatography, anion IE chromatography, mixed mode and anionic IE chromatography, and sterile filtration. Chromatography steps can be repeated.

The monoclonal antibody is linked to Sepharose CL-4B activated with cyanogen bromide. After completion of the antibody binding step, excess sites are blocked by reaction with glycine and the affinity resin is extensively washed. The resin is stored in Tris buffer with 0.01% thiomersal.

GENERAL AREAS OF DEFICIENCY (PHARMACEUTICAL) IN THE APPLICATION FOR A MARKETING AUTHORISATION

The Dutch Rapporteur has raised a number of pharmaceutical questions in their assessment report and these are generally endorsed. In addition to the Rapporteur questions, further questions are proposed by the UK secretariat. The list of Dutch questions and the proposed UK pharmaceutical questions are given at the end of the report and in the draft Biological subcommittee recommendations.

The major areas are:

- Drug Substance Specifications.
  Although the Company notes that the drug substance can not be fully characterised (because of post- translational modifications leading to heterogeneity and because of its large size) the specifications could probably be improved to include more physico-chemical characterisation.
  - Impurities. The proposed limits for some impurities (eg CHO proteins, BSA) appear high

- Bioassay: The assay needs to be cross validated against the Ph. Eur. method, and fiducial limits need to be applied.

- Anti FVIII C monoclonal antibody column (used in purification)
  - As two sites of manufacture of the MAb are proposed, further information is required to show comparability of the MAb produced at different sites. Specifications for the MAb may need to be improved.
  - It needs to be clarified whether the same MAb columns are used for Recombinate and Hemofil-M (a plasma derived FVIII).
  - Viral removal inactivation potential of the manufacturing process at one site (Hyland) needs to be indicated.

- Stability. The data supplied does not appear to support a shelf life of 2 years at 2 C to 25 C.

- A suitable name needs to be given to the drug substance (to distinguish it from other recombinant FVIIIs or native material)

Committee's advice is sought on whether the Company should be encouraged to incorporate a suitable a viral inactivation/removal step (eg heat, chemical inactivation, filtration etc) into the manufacturing process.

Other areas where further information or clarification is required includes genetics, cell banks, cell culture, purification, monoclonal antibody production and matrix manufacture, von Willebrands factor (co expressed with rAHF), impurities,batch analyses, drug substance and dosage form specifications, albumin, stability.

Viral Removal/inactivation

The drug substance, rAHF, is produced from a genetically engineered mammalian CHO cell line, and is the purification process involves a moncolonal antibody column. The MAb is obtained from a murine hybridoma.

2K

The genetically engineered CHO 10A1C6 cell line did not show the presence of viruses under the test methods except for A and C type retroviral particles (by electron microscopy). A scaled down purification process for rAHF has been studied for viral clearance (BVD, PI3, murine xenotropic retrovirus, Reo 3, SV 40) and clearance rates of 10E7 to >10E13 were obtained (see Pharm Exp Rep).
The murine hybridoma cell line did not reveal the presence of viruses except for type A and C retroparticle (electron microscopy) and was positive for manganese dependent RT activity in some tests.
The MAb can be made at two sites (Celltech and Hyland) . The viral clearance studies performed at Celltech (MLV, IBR, Reo3, SV40) gave a clearance of >10E6. The capacity of the process at Hyland to remove viruses has not been performed but the Company has been asked to investigate (see Q5 from the Rapporteur). Celltech and Hyland use different cell culture processes and purification procedures for the MAb.

Although the information on viral testing and clearance does not cause concern at this stage it may be i) difficult to fully predict or test cell banks for all viruses, or ii) to totally rely on the removal of viruses by partition effects in manufacturing processes. Therefore Committee's advice is sought on whether (as part of good manufacturing strategy) Companies who utilise mammalian cell lines should be encouraged to incorporate a suitable viral inactivation/removal process (eg heat, chemical inactivation, filtration etc).

PHARMACEUTICAL RECOMMENDATION

The pharmaceutical questions raised by the Dutch Rapporteur are endorsed. The UK raises additional questions. The Dutch questions and the proposed additional UK pharmaceutical questions are given in the Draft Recommendations of the Biological Subcommittee (July 1992)

Provided the Pharmaceutical questions are resolved a grant of a product license could be recommended.

E N Gate June 1992

SUBCOMMITTEE ON BIOLOGICALS

DRAFT RECOMMENDATIONS

Number
PL/0116/0233-5
(CPMP)

Company
Baxter Healthcare
Ltd

Product
Recombinate
250IU, 500IU
1000IU

Therapeutic Class
Clotting Factor

Active
Constituent
Antihaemophlic
Factor
(Recombinant)
[Company Term]

On the evidence before them, the subcommittee recommended that the following comments and points should be forwarded to the CPMP/Rapporteur:

i) Dutch rapporteur questions

The UK supports the pharmaceutical questions raised by the Dutch Rapporteur. These questions are appended.

ii) Additional UK pharmaceutical questions:

Part I

IA ADMINISTRATIVE DETAILS

1. It would be helpful if in section 1A3(d) the manufacturers of the monoclonal antibody and immunoaffinity resin could be indicated. (remark)

IB Summary of Product Characteristics

2. In the SPC, product literature, labels etc a suitable name should be used for the drug substance which distinguishes it from native Factor VIII and other recombinant Factor VIIIs. A suitable approved name for the drug substance should be applied for. (Major Point)

3. It would be helpful if the average specific activity for the drug substance was given in the SPC. (point for clarification)

PART IIB

MANUFACTURE OF THE DOSAGE FORM

4. The bioburden of the pre-sterile filtration solution should be monitored.

2 M

SUBCOMMITTEE ON BIOLOGICALS

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DRUG SUBSTANCE SPECIFICATIONS

5. The Company note that they have been unable to fully characterise the drug substance. The rationale for not including a range of physico-chemical tests (other than SDS-PAGE) into the drug substance specifications should be given and justfied. (major point)

6. Unless justified, the carbohydrate composition should be monitored. (major point)

7. The Company have given only a minimum potency in the drug substance specifications. The drug substance is a recombinant molecule with only low amounts of impurities. Therefore it would also be of concern if the drug substance was to become significantly more potent than that established. Suitable upper and lower limits for mean result and fiducial limits should be set. (point for clarification)

8. The result of the bioassay is given in units while the expression of strength of the dosage form is given in IU. the position should be resolved. (point for clarification).

CELL BANKS

9. It should be clarified whether both WCB K and P are used currently. The size and rate of use of cell banks should be clarified. It should be clarified whether there are any differences in the population doubling levels between the two cell banks K and P. (point for clarification)

CELL CULTURE

10. During cell culture the 2500L bioreactors are harvested about every three days until 65 population doublings. The usual duration of the cell culture process and the number of harvests should be indicated. (point for clarification)

2

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11. It is indicated that each 2500L bioreactor is harvested every three days, and that filtered conditioned media from several bioreactors is loaded onto the first chromatography resin as a single batch. Details on the storage of harvests should be given together with any limits on the length of storage of harvests or any affect of storage on stability. (point for clarification)

12. It should be indicated if there are any differences in the rAHF produced at different times during the cell culture process. (major point)

PURIFICATION

13. The Company (Baxter) also produces another Factor VIII preparation (Hemofil-M) which is purified using a monoclonal antibody column. Hemofil-M is derived from human plasma. It appears that the same murine hybridoma (F8.1.5.6) is referred to for Hemofil-M and Recombinate. In the proposed SPC for Recombinate it is claimed that "as Recombinate is produced by mammalian cell culture, no human viral agents should be present". Therefore:

13.1 It should be clarified whether the monoclonal antibody used to make the affinity column for use in the manufacture of Recombinate and Hemofil-M are in fact exactly the same. (point for clarification)

13.2 It should be confirmed that the manufacturing process for Recombinate does not share purification columns with those used for Hemofil-M, ie dedicated columns are used. It should be indicated whether any common facilities are used for Recombinate and blood derived products. 2(Major Point)

14. It should be confirmed that reprocessing in drug substance manufacture will not occur. (point for clarification)

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15. It is indicated (p464) that the donors for human transferrin used in MAb cell culture is tested for HTLV III and HBsAg. It should be indicated if testing includes antibodies for HIV 1 and 2, and HCV. The human transferrin should comply with the CPMP guidelines 'Medicinal products derived from human blood and plasma'. (point for clarification)

16. Brief details of the bioreactors used for monoclonal antibody production should be given. (point for clarification)

17. Details of the method for the rAHF inhibition assay referred to in the acceptance criteria (GI) on p504 and the Factor VIII function test cross referred to on p499 should be given (point for clarification)

18. The Company note that it is difficult to fully characterise the drug substance, rAHF. Therefore control of the method of manufacture is important to ensure consistency of the drug substance. The monoclonal antibody immunoaffinity column is a key step in the purification process. As two manufacturers of the MAb are proposed, the acceptance criteria for physico-chemical tests should be improved (rather than 'report result' or '>95%' etc) so that MAb from either site of manufacture is comparable. (This supplements the question from the Rapporteur asking for the results of a study of a direct comparison of material from the two sites). (major point)

19. At the moment the description of the manufacture of the immunoaffinity matrix is brief (reference is made on p503 to a flow diagram). The manufacture should be described. (major point)

20. If a different vendor is used for immunoaffinity matrix production (as mentioned on p452), the Marketing Authorisation should be varied. (remark)

21. The specifications for the F8.1 monoclonal antibody Sepharose CL-4B immunoaffinity resin should be improved to include a test (with suitable limits) to ensure that factor VIII does bind sufficiently to the column. (major point)

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CHARACTERIZATION OF THE DRUG SUBSTANCE

22. Figure IIC:1.2.6a:1-1 (SDS-PAGE of rAHF) referred to on p632 appears to be missing as do pages 633-4. Figure IIC:1.2.6c:2-1 (comparing rAHF and pdAHF on SDS-PAGE) referred to on page 715 appears to be missing from the data as do pages 717, pp722-3, pp735-6, pp750-1. This may not be an exhaustive list. These pages should be provided so that the characterization of the drug substance can be assessed. (point for clarification)

von WILLEBRAND FACTOR

23. The Company's production strategy uses genetically engineered CHO cells which coexpress rvWF (in order to stabilize the secreted rAHF). However, the structure of their rvWF has not been clearly given. It is not indicated whether the rvWF secreted from the cells has the amino acid sequence predicted from the gene sequence utilised or whether post translational modification occurs. It is not clear whether there are differences (eg amino acid sequence, glycosylation etc) between the rvWF produced and 'native' vWF. The position should be resolved (major point).

IMPURITIES

24. It should be clarified whether any rAHF related proteins (eg aggregates etc) or whether non rAHF proteins are seen on SDS-PAGE. If necessary appropriate tests with suitable limits should be introduced into specifications. (point for clarification)

25. The calculated clearance factors (p562) for some impurities does not appear to support the proposed limits eg p774. For example, the proposed limit for CHO proteins and BSA appears high (limits proposed 1ug and 0.8ug per 1000IU, assuming minimum specific activity of 4000IU/mg this is equivalent to about 0.4% and 0.32%). The position should be clarified. (major point)

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BATCH ANALYSES (DRUG SUBSTANCE)

26. The batch analyses for the drug substance given on pages 807-11 give the results of some tests as "meets specification" which makes it difficult to assess the consistency of the product. (point for clarification).

27. The confidence intervals for bioassay results should be given. (point for clarification)

Part IIE

DOSAGE FORM SPECIFICATIONS

28. The acceptance criteria for the immunoblot test is given in the specifications (IIE p826-7) as 'complies to specification'. The acceptance criteria should should be improved and should unless justified specify which bands may be present. (major point).

BATCH ANALYSES OF FINISHED PRODUCT

29. It was unclear from the batch analyses given on p838-9 what the nominal potency of the batches were. It is therefore difficult to assess whether the bioassay results were supportive of the proposed specification limits. Confidence limits for bioassay results were also not given. the position should be clarified. (point for clarification)

IIF

STABILITY OF THE DRUG SUBSTANCE

30. It should be indicated whether aggregates or degradation products have been seen on storage. It is unclear whether the acceptance criteria for SDS-PAGE or RP-HPLC tests would exclude bands or peaks not normally seen. As the results of stability studies (eg p851) are given as 'meets specification' or 'conforms' it is difficult to assess whether any degradation is seen. (point for clarification)

2

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STABILITY OF THE DOSAGE FORM

31. The data supplied so far does not clearly support a shelf life of 2 years at 25 C. The potency (appendix IIF:2.3 a-3) appeared to fall when stored at higher temperatures eg to below 90% at 25 C or 30 C. However, when stored at 5 C no real trend for loss of activity was seen. This infers that degradation at 25 C is significantly higher than at 5 C. This may be particularly important as the Company have noted that it is difficult to fully characterise the drug substance. The proposed shelf life should be reduced or justified. (major point)

32. Unless justified, it should be indicated whether aggregates or degradation products of rAHF are seen on storage at the proposed maximum temperature of storage. (major point)

33. The results of the immunoblot test in stability studies on the dosage form (appendix IIF:2.3 a-3 p14) are given as 'pass'. It should be indicated if any bands not normally seen are observed. (point for clarification)

PART IIQ

34. It should be confirmed that all sterile device components provided with the product comply with EEC requirements for sterile devices. Brief information on these devices should be provided, eg supplier, materials of which they are composed, methods of sterilisation and packaging etc. (point for clarification)

Part V

35. Appropriate samples of Recombinate should be provided.

REMARK TO BIOLOGICALS SUBCOMMITTEE

Although the information on viral testing and clearance for Recombinate does not cause concern at this stage it may be i) difficult to fully predict or test cell banks for all viruses, or ii) to totally rely on the removal of viruses by partition effects in manufacturing processes. Therefore Committee's advice is sought on whether (as part of good manufacturing strategy) Companies who utilise mammalian cell lines should be encouraged to incorporate a suitable viral inactivation/ removal process (eg heat, chemical inactivation, filtration etc).

25

Dutch Rapporteur's Pharmaceutical Questions, appended to Biological subcommittee Draft recommendations July 1992

CONCLUSION

The following objections are present:

**Part II C 1: Specifications and routine tests**

1. Potency assay: A requirement for the fiducial limit of error of the estimated potency is lacking and should be added to the specification (fiducial limits of error (p=0.95) of the estimated potency relative to the stated potency).

**Part II C 1: Genetic stability**

2. The stability of the cell culture during a common production run has been validated at the RNA and DNA level. For recombinant proteins it is common to demonstrate sequence-stability at both the DNA and the protein level.
The Expert Report states (page 36) "the product produces at 127 generations was indistinguishable from that produced at < 65 generations". Supporting data (analysis methods, results) of this statement could not be found in the dossier and -in order to support the stability of the produced protein sequence- are asked for.

**Part II C 1: Purification process**

3. The reuse criteria for chromatographic columns should be stated more precisely.
Limits for the duration of use and/or number of runs should be considered once more experienced has been gained.

**Part II C 1: Monoclonal antibodies used in immunoaffinity chromatography**

4. Monoclonal antibody batch analysis results of 3 Celltech batches and 5 Hyland batches are submitted. However, a conclusive comparison of these results is not possible due to differences in in-house analysis (Celltech vs. Hyland) methods and (presumably) reference preparations.
In order to ensure biological equivalence of the monoclonal antibodies produced by both processes comparative results of batches produced by both processes should be provided, e.g. direct comparative analysis of batches from both processes on the same SDS-PAGE and (narrow pH-gradient) IEF gels (submit clear photographs) and quantitative results of r-AHF binding studies.

5. The in-process testing of the Hyland F8.1 production process should be described in more detail. It is unclear which in-process tests on microbial and viral contamination are performed during each production process. Moreover the capacity of the Hyland F8.1 purification process to remove/inactivate potential viral impurities should be adressed.

**Part II C 2: Human albumin solution**

6. In addition to the quality reference 'Ph.Eur. monograph' it should be stated more explicitly which virus tests will be carried out on blood donations. In this aspect the albumin ought to comply with the CPMP Note For Guidance 'Medicinal products derived from human blood and plasma' (coming into operation 1 may 1992).

Part II E

7. Potency specification: The requirement for the fiducial limits of error of the assay should be added to this specification.

8. The application of an one-stage assay in the potency determination of Recombinate should be justified by means of cross validation data (several batches) against the Ph.Eur. two-stage assay (using the MEGA 1 standard).

PL 00016 / 0233-35 Recombinate (Recombinant Factor VIII)

Preclinical Data Assessment Dr R M Lee May 1992

## 1. Introduction

Recombinate is the product of Chinese Hamster cells derived from the ovary and programmed genetically to express human Antihaemophilic Factor (VIII) for the treatment of X-chromosome linked bleeding disorder.

In some haemarthrosis treatments, the amount of Factor VIII administered intravenously (25 IU/kg) is relatively low and designed to achieve approximately 50% of normal circulating blood level.

In other more serious cases, the Factor VIII is to be given repeatedly with the object of reaching 100% of normal concentration, but avoiding excess by monitoring plasma AHF levels.

Treatment would usually continue for 3 days, except in the most severe bleeds, when there is no time limit. Dose level may be appropriately increased to overcome inhibitors at relatively low concentration.

Intrinsically there are no safety concerns associated with the use of a human blood factor as replacement therapy; however, the route to this synthetic material is complex and toxicity testing has been performed throughout the development process to reassure that no adventitious agents have been introduced (or have been removed after introduction).

Thus, several of the reported studies relate to products from cell lines and sites of production which were examined before the final selection of cell line 10A1C6 and the "Andover" manufacturing site.

The pharmaco-toxicological Expert Report (Appendix 1) follows the standard CPMP format with tabulation of the individual studies. Only the relevant tables are attached to this assessment i.e. those which refer specifically to the product intended for clinical use.

## 2. Pharmacodynamics

Effectiveness of this rAHF material was tested in dogs displaying an inherited haemophilia due to Factor VIII deficiency and shown to be active with a normal half-life, as long as proteolysis was inhibited by the inclusion of aprotinin in the preparation.

Doses up to 5 or 10 times the expected maximum human dose were tested for other pharmacological activity in rodents and dogs. Apart from shortening of APTT (an expected consequence of excess Factor VIII) there was no significant effect on major organ systems.

3. Kinetics and Biotransformation

Distribution of rAHF was also studied in haemophilic dogs (only 2 because of the scarcity of such models). The biological (activity) half-life (ca. 18 hours) was comparable to that of plasma derived Factor VIII; in rats, $^{125}I$ labelled rAHF material showed that the radioactivity remained largely in the blood for similar periods, with the $t_{1/2}$ and AUC little affected by repeated administration.

Since there is no information on the in vivo stability of $^{125}I$-rAHF, the presence and retention of radioactivity in tissues at various times after administration is difficult to interpret. The Applicant's suggestion is that there is an initial metabolism of the rAHF to lower molecular weight substances, but that this is concentration dependent, leaving some active material in the blood for usefully long periods. Some iodine is attached to components of low enough weight to be accumulated by the thyroid.

For these rat studies there is no comparison with radiolabelled pdAHF, but the distribution and elimination after i.v. dosing of rAHF does not appear to raise any concerns for safety or effectiveness in repeated clinical administration.

4. Toxicology

The single dose studies with the material intended for the final product (BL 160 lot No. 2938R009) tested a maximum 5000 IU/kg i.v. and 10,000 IU/kg subcutaneously and orally in rats. No acute effects attributable to the treatment were observed over 14 days.

I.v. administration of 2,500 or 5,000 IU.kg of the same material to 1 male and 1 female beagle caused a reddening of the skin and mild oedema, together with slight change in behaviour for a few hours after dosing. Further observation for 14 days revealed no other signs of adverse effect.

Repeated dosing to rats and cynomolgus monkeys also indicated no toxicity beyond that to be expected as a result of antibody formation to a product containing such foreign proteins, viz :- a prolongation of activated partial thromboplastin time (APTT).

The maximum doses administered daily for 1 month in these tests (1,000 IU/kg for rats and 500 IU/kg for monkeys) were 10 or 5 times in excess of the dose proposed for use in the clinical indication. In the 4 week monkey study, dosing of rAHF produced no antibodies to BSA, Chinese Hamster Ovary protein or von Willebrand factor, but antibody to the last-named was found in the comparator control group of animals receiving pdAHF.

The general safety testing thus confirms the non-toxic nature of the rAHF itself and supports the purification procedure by indicating that effects due to residues are not present in the batches from the selected cell line and manufacturing facility.

The Expert Report argues that Reproduction Toxicity studies are not necessary because this rAHF product will be Category C for use in pregnancy (i.e. only to be given "if clearly needed") and the disease indicated for treatment occurs predominantly in the male human population.

Such an argument takes no account of the risk, if any, to the male reproductive system. No histological changes in testicular cells were found in the 1 month studies, but this is incomplete reassurance since the testis weights after 4 weeks' treatment of monkeys at 250 and 500 IU/kg were >50% higher than controls (vehicle or pdAHF) in some animals. (p 6)

These weight changes resolved in monkeys of the 2 week recovery group and were not seen in male rats under similar exposure conditions.

The applicant has adopted a defensive strategy of conducting Mutagenicity studies to cover the possibility that unidentified process contaminants may pose a risk. No genotoxicity was detected in vitro or in vivo.

Carcinogenicity studies were not attempted, antibody formation and the lack of mutagenicity being cited as adequate reasons. This is accepted.

A particular study of the Antigenic properties of B160 rAHF was made and the results, considered in the Expert Report, lead to the conclusion that rAHF will be similar to the already available pdAHF in this respect.

Local tolerance by veins after i.v. injection was examined during the single dose studies and found to be satisfactory. No tests of perivenous or intra-arterial administration were done.

## CONCLUSION and RECOMMENDATION

"Recombinate" Factor VIII product was not distinguishable from plasma derived product in terms of physiological action at normal or excessive blood levels. No unexpected adverse events of significance resulted from the administration of this rAHF to animal models, but not all, possibly relevant, tests were performed.

On balance, it is recommended that, in combination with the purification process, sufficient preclinical safety testing has been completed to permit the Grant of a Product Licence on the basis of evidence that this recombinant material is not significantly different from other AHF derived from more direct human sources.

TESTIS WEIGHTS

Table 011-1

Influence of BL-160 on organ weight in male cynomolgus monkeys

Exp.No. : SBL73-37

Stage : End of drug administration

| Anim.No. \ Item / Unit | Pituit. mg | Thyro.R g | Thyro.L g | Adre.R g | Adre.L g | Testi.R g | Testi.L g | Pancr. g | Thymus g | Subm.R g | Subm.L g | Spleen g |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control-1 0 (U/kg) | | | | | | | | | | | | |
| 1 | 78 | 0.21 | 0.21 | 0.26 | 0.39 | 14.5 | 14.8 | 7.2 | 2.2 | 1.5 | 1.7 | 7.4 |
| 2 | 77 | 0.16 | 0.21 | 0.28 | 0.37 | 18.2 | 18.3 | 7.0 | 1.8 | 1.4 | 1.1 | 7.6 |
| 3 | 69 | 0.17 | 0.18 | 0.30 | 0.21 | 12.9 | 12.9 | 6.3 | 1.4 | 0.9 | 0.9 | 13.0 |
| Mean | 74.7 | 0.180 | 0.200 | 0.280 | 0.323 | 15.20 | 15.33 | 6.83 | 1.80 | 1.27 | 1.23 | 9.33 |
| ±S.D. | 4.9 | 0.026 | 0.017 | 0.020 | 0.099 | 2.72 | 2.74 | 0.47 | 0.40 | 0.32 | 0.42 | 3.18 |
| BL-160 125 (U/kg) | | | | | | | | | | | | |
| 7 | 85 | 0.42 | 0.46 | 0.26 | 0.16 | 13.0 | 12.4 | 5.6 | 1.0 | 0.9 | 0.8 | 6.2 |
| 8 | 87 | 0.25 | 0.28 | 0.34 | 0.24 | 16.3 | 15.1 | 5.7 | 2.2 | 1.6 | 1.9 | 9.2 |
| 9 | 65 | 0.24 | 0.22 | 0.22 | 0.28 | 13.9 | 13.7 | 5.1 | 1.1 | 0.8 | 0.8 | 8.7 |
| Mean | 79.0 | 0.303 | 0.320 | 0.273 | 0.227 | 14.40 | 13.73 | 5.47 | 1.43 | 1.10 | 1.17 | 8.03 |
| ±S.D. | 12.2 | 0.101 | 0.125 | 0.061 | 0.061 | 1.71 | 1.35 | 0.32 | 0.67 | 0.44 | 0.64 | 1.61 |
| BL-160 250 (U/kg) | | | | | | | | | | | | |
| 13 | 84 | 0.21 | 0.23 | 0.26 | 0.43 | 16.4 | 15.7 | 4.9 | 1.7 | 2.8 | 2.9 | 7.8 |
| 14 | 71 | 0.20 | 0.24 | 0.39 | 0.26 | 19.7 | 20.3 | 9.2 | 1.1 | 1.5 | 1.3 | 21.7 |
| 15 | 76 | 0.32 | 0.33 | 0.27 | 0.35 | 27.1 | 23.3 | 7.4 | 0.8 | 1.8 | 2.0 | 16.0 |
| Mean | 77.0 | 0.243 | 0.267 | 0.307 | 0.347 | 21.07 | 19.77 | 7.17 | 1.20 | 2.03 | 2.07 | 14.83 |
| ±S.D. | 6.6 | 0.067 | 0.055 | 0.072 | 0.085 | 5.48 | 3.83 | 2.16 | 0.46 | 0.68 | 0.80 | 6.95 |
| BL-160 500 (U/kg) | | | | | | | | | | | | |
| 19 | 102 | 0.43 | 0.39 | 0.33 | 0.51 | 19.5 | 19.7 | 4.7 | 1.4 | 1.7 | 1.6 | 12.4 |
| 20 | 70 | 0.19 | 0.20 | 0.40 | 0.28 | 23.2 | 22.0 | 6.0 | 1.1 | 1.6 | 1.5 | 8.3 |
| 21 | 67 | 0.37 | 0.34 | 0.35 | 0.28 | 18.7 | 18.4 | 5.8 | 1.2 | 1.3 | 1.3 | 10.1 |
| Mean | 79.7 | 0.330 | 0.310 | 0.360 | 0.357 | 20.47 | 20.03 | 5.50 | 1.23 | 1.53 | 1.47 | 10.27 |
| ±S.D. | 19.4 | 0.125 | 0.098 | 0.036 | 0.133 | 2.40 | 1.82 | 0.70 | 0.15 | 0.21 | 0.15 | 2.06 |
| Control-2 500 (U/kg) | | | | | | | | | | | | |
| 29 | 84 | 0.29 | 0.27 | 0.21 | 0.31 | 2.9 | 4.5 | 7.5 | 1.5 | 1.6 | 1.8 | 10.0 |
| 30 | 74 | 0.29 | 0.24 | 0.21 | 0.26 | 9.3 | 9.6 | 7.8 | 3.0 | 1.9 | 1.7 | 6.5 |
| 31 | 71 | 0.60 | 0.56 | 0.30 | 0.32 | 16.6 | 18.2 | 4.7 | 3.6 | 2.1 | 1.6 | 8.3 |
| Mean | 76.3 | 0.393 | 0.357 | 0.240 | 0.297 | 9.60 | 10.77 | 6.67 | 2.70 | 1.87 | 1.70 | 8.27 |
| ±S.D. | 6.8 | 0.179 | 0.177 | 0.052 | 0.032 | 6.85 | 6.92 | 1.71 | 1.08 | 0.25 | 0.10 | 1.75 |

Influence of BL-160 on organ weight in male cynomolgus monkeys

Stage : End of recovery

| Anim.No. \ Item / Unit | Pituit. mg | Thyro.R g | Thyro.L g | Adre.R g | Adre.L g | Testi.R g | Testi.L g | Pancr. g | Thymus g | Subm.R g | Subm.L g | Spleen g |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BL-160 500 (U/kg) | | | | | | | | | | | | |
| 22 | 71 | 0.26 | 0.22 | 0.34 | 0.45 | 15.4 | 14.1 | 7.3 | 4.5 | 1.7 | 1.8 | 13.1 |
| 23 | 68 | 0.31 | 0.29 | 0.25 | 0.31 | 15.4 | 15.9 | 6.8 | 2.6 | 1.4 | 1.2 | 14.5 |
| Mean | 69.5 | 0.285 | 0.255 | 0.295 | 0.380 | 15.40 | 15.00 | 7.05 | 3.55 | 1.55 | 1.50 | 13.80 |

MEDICAL ASSESSMENT

This is a CPMP Biotech application for Recombinate - the first for a recombinant Factor VIII. The Netherlands are rapporteur.

Recombinate is indicated for:

"Acquired and congenital Factor VIII deficiency for the prevention and control of haemorrhagic episodes."

In the indications section the company state that "as Recombinate is produced by mammalian cell culture, no human viral agents should be present, therefore it may be justified to consider it as a primary treatment option in patients not previously exposed to human blood derivatives. As expected no evidence of human viral infection has been reported with the use of Recombinate".

Claims are also made for use when large repeated doses are required (absence of blood group specific antigens), and patients with inhibitors below 10 Bethesda Units/ml.

Recombinate is not indicated for von Willebrand's disease.

The dosage is tailored to the individual patient and the type of bleed. Instructions are at page 82. These doses are those used with standard Factor VIII concentrates.

1. Introduction

This recombinant Factor VIII was developed by Genetics Institute in Boston.

The protein is expressed in Chinese Hamster Ovary (CHO) cells. The original cell line VIII-49, only expressed the gene for Factor VIII. A small amount of product was produced in roller bottles at the Summa plant in Alburquerque, New Mexico. It is called Summa product.

The Summa product was given to the first 2 patients to receive Recombinate for a pharmacokinetic study.

Subsequently, presumably to increase yield a new cell line 10A1C6 was developed. This cell line contains a double construct with genes for both Factor VIII and von Willebrand Factor (vWF).

The vWF secreted into the culture medium stabilises the Factor VIII and presumably allows higher yields. This also means that a serum free medium can be used.

Affinity chromatography using a Factor VIII monoclonal antibody is used for downstream processing and the vWF is lost during the process.

Three different production procedures for the 10A1C6 cell line have been carried out, all at the Genetics Institute plant in Andover, Mass.

Batches from the 10A1C6 cell line ("Andover" batches) have been used in all but the first two patients.

Recombinant Factor VIII is glycosylated, but presumably differently to the native product. However since the majority of O-glycosylations on Factor VIII are in the B domain, which is clipped by thrombin during activation, these differences may not be important.

Recombinate contains plasma derived human albumin to stabilise the product.

## 2. Clinical Studies

A total of 126 patients have been exposed to Recombinate of whom 2 received the Summa product and the remainder material prepared at Andover. This includes 58 previously untreated patients.

Some patients have received up to 4 years treatment.

4 clinical studies are reported of which two are pivotal: long term treatment in previously treated haemophiliacs, and a study in previously untreated patients (pups). In addition there is a pharmacokinetic study in 2 patients and a small study from Japan.

### 2.1 Pharmacokinetics (p 54)

Recombinate has been compared to two Baxter Products - Haemofil T (standard heat treated concentrate) and Haemofil M (monoclonal purified concentrate).

Kinetics were looked at after the first dose and at regular intervals during long term studies.

Half life and recovery (traditional methods of monitoring Factor VIII) were similar to the standard products.

The T½ of 16hrs is comparable to the data for other Factor VIII products.

Long term treatment did not alter the kinetics of Recombinate.

### 2.2 Efficacy p 59

In the long term study patients treated themselves at home and few reported formally on efficacy.

For those patients who did, responses were termed good or excellent (p 59 ).

A good indicator of efficacy is that the majority of bleeds responded to a single infusion of Recombinate.

In the Japanese study (7 patients) (p 74), descriptive responses were provided.

It appeared that Recombinate was very effective.

In addition (p 60 ) a number of patients had surgery under Recombinate cover, successfully.

This includes one haemophiliac who had a liver transplant, surely a severe test of haemostasis.

2.3 Safety

2.3.1 Adverse events (p 61 )

Only a few minor adverse events were reported in the clinical trials. No deaths or withdrawals were associated with adverse events.

2.3.2 Liver function (p 61 )

LFT's were reported for all the previously treated patients. There was a tendency for fluctuation, as practically all the patients had long standing hepatic impairment.

One patient appears to have become HBsAg positive during treatment. However he had some markers of liver disease (HBcAb) at baseline.

Liver function tests on the pups were not reported. These results must be seen prior to licensing.

2.3.3 Inhibitors (p 68/71)

Prior to the advent of AIDS, the development of an inhibitor was the most serious complication for haemophiliacs. Mortality and morbidity from haemorrhagic events is markedly increased in these patients.

The incidence of inhibitors in patients with severe haemophilia in the UK is about 15%.

Most inhibitors appear early in treatment - 62% in less than 50 exposure days.

There has been concern about the possibility of an increased incidence of inhibitors in patients receiving Recombinate or highly purified plasma derived Factor VIII. Rates of up to 30% have been reported in pups receiving Monoclate (monoclonal purified VIII) or Kogenate (another recombinant Factor VIII product).

Patients receiving Recombinate have been carefully monitored for inhibitors and this aspect was the primary endpoint sought in the pup study.

No new inhibitors have developed in previously treated patients.

7 pups have developed inhibitors, including 3 of high titre. This is an incidence of 16% and is comparable to the baseline incidence in new patients.

The company will continue to monitor the situation.

2.3.4 T cell subsets (p 68)

These have been monitored in the long term study, where most of the patients were HIV positive.

There has been a tendency for a fall in $T_4$ count.

However individual data show marked fluctuation in patients over time.

Recombinate does not appear to affect T cell numbers.

3. Expert Report (p 75)

This is by Dr Ed Gompertz who is an employee of Baxter. It has a good discussion - particularly in relation to inhibitors.

The conclusions are appended.

4. Spc (p 81)

The statement that preclinical studies have shown that Recombinate is safe and effective should be removed from the sections on Undesirable Effects and Pharmacological properties.

5. Medical Comment

Recombinate is effective in the requested indications.

Data for LFTs in the pup study should be supplied to complete the safety package.

A product licence should be granted.

APPENDIX - 1

# IC: PHARMACOLOGY/TOXICOLOGY EXPERT REPORT

## INTRODUCTION

Individuals with classic hemophilia, an X-chromosome-linked bleeding disorder, are deficient or lacking in factor VIII (Antihemophilic Factor), a protein which participates in the intrinsic pathway of blood coagulation (1). Human and animal sourced therapeutic commercial concentrates of factor VIII have been on the market since 1965 and these products have dramatically improved the ease and outcome of treatment, however they have led to patient exposure to viral pathogens (e.g. hepatitis and AIDS [2,3,4]) and other harmful contaminants. Consequently there is a demand for therapeutic factor VIII concentrates with increased purity, activity, stability, and reduced viral risk (5,6). In response to this need, a manufacturing process has been developed to produce a human factor VIII derived from recombinant DNA technology, a recombinant form of antihemophilic factor (rAHF). This rAHF is the active ingredient in Recombinate™ Antihemophilic Factor (recombinant). Production of rAHF is not dependent upon plasma supply or processing, therefore the assurance of availability is greatly increased. It is also free of blood group antigens, thus potentially increasing the safety of large or repeated infusions (7). For additional information on the manufacturing process see Part IIC:1.2.3 Manufacturing.

The active ingredient, bulk Antihemophilic Factor (recombinant) is derived from culture supernatant of a Chinese Hamster Ovary (CHO) cell line genetically engineered to synthesize human Antihemophilic Factor. The secreted rAHF is purified from the cell culture medium by immunoaffinity chromatography utilizing an anti-factor VIII monoclonal antibody followed by ion exchange chromatography. Preclinical studies evaluated in this report were intended to demonstrate product safety and efficacy. In addition, rAHF manufactured at three facilities from two cell lines (VIII-H9 and 10A1C6) was compared with plasma-derived Antihemophilic Factor (pdAHF).

This report provides a critical evaluation of the preclinical experimental studies performed to assess the pharmacodynamic, pharmacokinetic, and antigenic properties of rAHF, as well as both the acute and subacute toxicity of this product. Ideally for a product such as factor VIII, which is intended for long term repeated use, an assessment of chronic toxicity would also be provided. However, as this rAHF is a human protein the assessments in animals of chronic toxicity is particularly problematic because, as a human protein, it will be antigenic in animals. The value of animal pharmacodynamic, pharmacokinetic and antigenicity studies is also somewhat suspect for the same reason, however provided these studies are interpreted as being only indicative of the probable metabolism and biological activity of the protein in man, they are useful.

With these caveats, the experiments providing the basis for this report are summarized in Table 1 and discussed below.

# Table 1. Summary of Studies

## Summa/Endotronics Material

| Study Number* | Species | Type of Study | Tabulated Study Report Page Number | Reference |
|---|---|---|---|---|
| PT-002 | Ferrets | Single Dose Toxicity | 92 | IIIA:1.1 |
| PT-001 | Rats | Single Dose Toxicity | 93 | IIIA:1.2 |
| PT-017 | Dogs | Efficacy/Pharmacodynamics | 111 | IIIF:1.1 |
| PT-018 | Dogs | Efficacy/Pharmacodynamics | 112 | IIIF:1.2 |
| PT-019 | Dogs | Pharmacokinetics | 118 | IIIG:1 |
| N/A | Mice | Antigenicity | 127 | IIIQ:1 |

## Genetics Institute - Cambridge, MA Material

| Study Number* | Species | Type of Study | Tabulated Study Report Page Number | Reference |
|---|---|---|---|---|
| PT-004 | Ferrets | Single Dose Toxicity | 94 | IIIA:2.1 |
| PT-003 | Rats | Single Dose Toxicity | 95 | IIIA:2.2 |
| PT-005 | Rats | Single Dose Toxicity | 96 | IIIA:2.3 |
| PT-021 | Dog | Repeated Dose Toxicity | 100 | IIIB:1 |
| PT-020 | Dogs | Efficacy/Pharmacodynamics | 113, 114 | IIIF:2.1 |
| N/A | Mice | Antigenicity | 128 | IIIQ:2 |

## Genetics Institute - Andover, MA Material

| Study Number* | Species | Type of Study | Tabulated Study Report Page Number | Reference |
|---|---|---|---|---|
| PT-006 | Rats | Single Dose Toxicity | 97 | IIIA:3.1 |
| PT-008 | Rats | Single Dose Toxicity | 98 | IIIA:3.2 |
| PT-009 | Dogs | Single Dose Toxicity | 99 | IIIA:3.3 |
| PT-010 | Rats | Repeat Dose Toxicity | 101, 102 | IIIB:2 |
| PT-015 | Monkeys | Repeat Dose Toxicity | 103, 104 | IIIB:3.1 |
| PT-023 | Monkeys | Repeat Dose Toxicity | 105, 106 | IIIB:3.2 |
| PT-011 | Bacteria | Mutagenicity | 107, 108 | IIID:1.1 |
| PT-012 | CHO Cells | Mutagenicity | 109 | IIID:1.2 |
| PT-013 | Mice | Mutagenicity | 110 | IIID:2 |
| PT-016 | Rats, Mice, Guinea Pigs | Pharmacodynamics | 115, 117 | IIIF:3.1 |
| PT-014 | Dogs | Pharmacodynamics | 116 | IIIF:3.2 |
| PT-007 | Rats | Pharmacokinetics | 119-125 | IIIG:2 |
| N/A | Mice | Antigenicity | 129 | IIIQ:3 |

* Study numbers for complete reports found in the Pharmacology/Toxicology Addenda are preceded with 98002; antigenicity studies are fully documented in Part IIIQ.