

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

**Center for Biologics Evaluation & Research - Food & Drug Administration**

Laboratory of Immunobiology, Division of Monoclonal Antibodies, HFM-564

NIH Campus, Building 29B, Room 3NN10

1401 Rockville Pike, Rockville MD 20852

Telephone (301) 8270808

Facsimile (301) 8270582

**Product Review**

**Date:** December 4, 1997  
**From:** Barbara Rellahan - HFM-564  
Laboratory of Immunobiology, Division of Monoclonal Antibodies, CBER  
**To:** BLA  
**Through:** Acting Chief, LI, Division of Monoclonal Antibodies  
Director, Division of Monoclonal Antibodies  
Office of Therapeutics Research and Review  
**Subject:** BLA 97-0736  
Date of submission: June 6, 1997  
Date received by FDA: June 6, 1997  
Date received by DMA:  
Decision date: December 10, 1997

**Therapeutic Agent:** Recombinant humanized IgG1 Anti-Tac (HAT) or ZENAPAX (daclizumab). Roche code Ro 24-7375, WHO # is 7164.

**Manufacturer:**

Hoffmann-La Roche  
Building 28, Multi-Product Facility  
340 Kingsland Street  
Nutley, New Jersey, USA

**Clinical Indication(s):** Prevention of acute renal allograft (cadaveric) rejection.

**Objective:** Use as an low toxicity, adjunct to standard immunosuppressive therapy in humans.

**Product Rational:** To block the 55-kD alpha chain of the IL-2 receptor on activated CD25+ lymphocytes but not to affect unactivated lymphocytes.

**Study Objective(s):**

Phase: Biologic Licensing Application

**Cross Referenced IND(s):** DMF (b) (4) (compositions and properties of glasses used for bottling), DMF (b) (4) (Glass Products), DMF (b) (4)

(b) (4)

## Index

Background .....	3
Development .....	3
Daclizumab Expression Vector p1H4.45.4. ....	5
Cloning of Stable Transfectant (b) (4) .....	6
(b) (4) Cell Banks .....	8
End-of-Production Cells/Extended Cell Bank .....	10
Manufacturing .....	13
Drug Product .....	22
Product Stability Studies .....	26
Reference Standard .....	27
Conclusions .....	31
Recommendations .....	35
Appendix I (Comparability Studies) .....	36
Appendix II (Validation Studies) .....	39
Appendix III (Assay Validations) .....	45

I have reviewed the production and manufacturing contained in volumes 1 to 18 of the original Hoffman-LaRoche BLA submission, dated June 6, 1997 concerning the product license application for Daclizumab (Zenapax®). In addition I have reviewed an amendment to the BLA dated 8/22/97, IND (b)(4) amendment 40 and four responses to questions and issues from teleconferences with Hoffman-LaRoche on August 15 and 21, 1997 and issues raised during the pre-licensing inspection. These responses were received by the FDA on 9/11/97, 9/24/97, 11/3/97 and 11/26/97. The following is the summary of the rationale and a brief report of my review.

## **Background**

**Mechanism of Action:** Daclizumab blocks binding of IL-2 to the IL-2R on activated lymphocytes. It can also mediate ADCC through its Fc region leading to the specific clearing of activated lymphocytes. Patients treated with daclizumab had increased serum levels of soluble IL-2R $\alpha$ , which may be due to mAb binding to the soluble receptor and increasing its half life. Activated T cells become saturated with mAb by 10 hours post infusion, and remain saturated for up to 64 days when given a 5.0mg/kg infusion. With multiple infusions, the CD25 receptor can remain saturated for up to 120 days. The binding does not appear to lead to a rapid clearance of cells, although there is a modest decrease in the number of CD25+ (IL-2R) cells.

**Structure and Specificity:** Daclizumab is a recombinant humanized monoclonal antibody, subclass IgG1. The molecule is composed of two identical heavy chain subunits and two identical light chain subunits. The molecule is approximately 90% human origin and 10% murine origin. It contains ~ 2% carbohydrate. The molecular weight of the heavy chain subunit is 48,864 and for the light chain subunit it is 23,210. That of the whole molecule is 150,000 dalton. The molecular formula for the molecule is C<sub>6398</sub>H<sub>9860</sub>O<sub>2012</sub>S<sub>44</sub>.

The activity of the monoclonal produced from the original murine hybridoma clone was determined to be reactive with human T cells activated by PHA, Con A, PWM, SLO and allogenic cells but not with fresh peripheral blood T cells, B cells, monocytes, thymocytes and B cells activated by EBV or pokeweed mitogen. It was later determined that the monoclonal antibody blocks the binding of purified TCGF (IL-2) to CTC cells and that it also inhibits TCGF induced proliferation of CTC cells. It was further demonstrated that the monoclonal in some way blocks the interaction of IL-2 and the IL-2R on human peripheral blood lymphocytes. Subsequent work showed that the antibody directly and competitively bound to the p55 subunit of the IL-2R with an affinity in the range of (b)(4).

## **Development:**

19 Page(s) has been Withheld in Full as b4 (CCI/TS) immediately following this page

## Drug Product

### Zenapax® Formulation

5 mg/ml (Concentrate = 25 mg/ 5ml) in 67 mM phosphate buffer, 0.02% polysorbate 80, (b) (4) NaCl. See vol 17:90 for detailed description of Manufacturing procedure of the final concentrate product.

<u>Ingredients</u>	<u>Amounts per ml</u>
Ro 24-7375	5 mg
Polysorbate 80 NF	0.20 mg
Sodium Chloride	4.60 mg
Sodium Phosphate Monobasic Monohydrate	3.588 mg
Sodium Phosphate Dibasic Heptahydrate	10.991 mg
Sodium Hydroxide	q.s. pH 6.9 +/- 0.1
Hydrochloric Acid	q.s. pH 6.9 +/- 0.1
Water for Injection	q.s. 1.0 ml
Vial volume	5.4 ml
Density	(b) (4)
Osmolarity	(b) (4)
pH	6.9 +/- 0.1

U.S.P. type I (b) (4) glass container having a nominal capacity fo 5 ml, fitted with a 13 mm (b) (4) rubber serum closure, held in place by an aluminum/plastic flip-off seal. The vials will be closed with (b) (4) stoppers. See vol. 18: 95 for validation of stopper extractables (b) (4) (b) (4) and extraction injection into mice and rabbits (page 117-121), Validation of container closure integrity (page 124).



## Conclusions

Listed below are issues which were identified during the course of my review that needed to be addressed by the manufacturer. These issues were communicated to Hoffman-LaRoche in two teleconferences on August 15 and 21, 1997. Any issues which were still outstanding with the company at the time of the pre-licensing inspection or that arose during the course of the inspection were also reviewed with the company at the inspection close-out meeting on October 10, 1997. In bold type I have summarized the response of Hoffman-LaRoche to each item.

1. The consistency data for the manufacture of three bulk protein lots (015126, 016017, 017027) is given in vol. 13:41. The consistency data indicate that the endotoxin concentration of the bulk product for all three lots is <1.25 EU/mg. On vol. 16:215, the lot release spec sheet for lot 017027 has the endotoxin concentration as none detected. Please comment on why are there values different. **During the inspection I was informed that these differences were due to the computer programs used in the different labs. One of the programs couldn't indicate a value as being (b) (4) and as this was the limit of sensitivity of the assay performed the value was recorded as 0 EU.**

2.

(b) (4)

(b) (4)

3.

(b) (4)

(b) (4)

4. Please be informed that stability studies need to be conducted on final-filled product, using the container and closure configuration intended for distribution, as well as on, unpurified bulk, and purified bulk product. Studies need to be conducted on product stored in upright and inverted positions. Please submit the data from these studies as soon as they become available. **Data was submitted as an amendment to the BLA on**

8.22.97. Indicated that the improved NSO material are stable at (b) (4) (drug substance), and 2-8°C (drug product) for (b) (4) months. Six month stability data was submitted on 11.12.97.

5. Please supply information on how Hoffman-LaRoche personnel conducted the viral clearance studies once the virally spiked preparations were received from (b) (4). Please specifically address what was used as the test articles which were spiked with virus prior to evaluation in each individual process step.  
Submitted 9.10.97.

6. (b) (4)  
(b) (4)

7. The lot release specifications for the IL-2R binding assay and the bioassay for the drug product have (b) (4) protein concentration ranges. We request that you commit to revise (b) (4) these ranges as your manufacturing experience increases.  
Commitment submitted 9.10.97.

8. Please comment on the differences between the sequences for the heavy chain gene of daclizumab (four nucleotide differences, one of which results in an amino acid change)  
(b) (4)

9. (b) (4)  
(b) (4)

[Redacted] (b) (4)

10. [Redacted] (b) (4)  
[Redacted] (b) (4)

11. Please be informed that you will need to submit stability data on all product intermediates. Issue address during the inspection and I have a copy of the validation data. [Redacted] (b) (4)  
[Redacted] (b) (4)

12. [Redacted] (b) (4)  
[Redacted] (b) (4)

13. Need to see data on all leachables from storage containers and bags. During the inspection [Redacted] (b) (4)  
[Redacted] (b) (4)

14. At the time of the inspection HLR was informed that in the absence of established column lifetime limits, they need to revise their column lifetime monitoring to include

column performance measurements. [REDACTED] (b) (4)

[REDACTED] (b) (4)  
[REDACTED] (b) (4)  
**(b) (4) This was addressed in a response to the agency dated 11.3.97 and is reviewed in the BLA under column maintainance.**

15. At the time of the inspection HLR was informed that they needed to submit data demonstrating that the LAL assay used to detect endotoxin has been validated against a [REDACTED] (b) (4) test. **Submitted in the 11.3.97 response and reviews in the BLA assay validation appendix.**

16. At the time of the inspection HLR was asked to revise their shipping carton label to include information on validated shipping conditions.

17. [REDACTED] (b) (4)

18. Please provide SOPs for in process specifications for the growth rate and viability of [REDACTED] (b) (4) **Submitted 9.10.97.**

19. Please provide an SOP for how you plan to determine when to begin the production of a new Working cell bank and how this will be carried out. **Submitted 9.10.97.**

20. Please submit a [REDACTED] (b) (4) validation for the soluble IL2-R used in the IL-2R ELISA assay and for the [REDACTED] (b) (4) cell line used in the Bioassay. **Submitted 9.10.97.**

21. Please provide an acceptance criteria for the viability of the [REDACTED] (b) (4) to be used in the Bioassay. **In a response to the agency dated 11.3.97, Hoffman-LaRoche stated they are still in the process of setting a limit for viability, but an interim limit of [REDACTED] (b) (4) viability is being used.**

### Recommendations:

The data submitted in this application support the conclusion that the manufacture of Daclizumab is well controlled and leads to a product that is pure and potent. The product is free from endogenous or adventitious infectious agents in a way that meets or exceeds the parameters recommended by the FDA. Manufacturing conditions have been validated by the use of adequate standardized methods and show that a consistent product is obtained in different production runs, maintaining the biochemical and biophysical properties of the product. Listed below are several issues which still need to be addressed. If they can be addressed adequately, I recommend approval of this product for human use.

1) As discussed during the pre-licensing inspection of the Nutley Hoffman-LaRoche manufacturing facility, please submit a revision to your shipping carton label to include information on validated shipping conditions.

2) Please submit an SOP on how you plan to validate new reference standards for the (b) (4) lot release specification assay. In your 'Protocol for the validation of the LC-MS/MS limit test for (b) (4) daclizumab drug substance' under preparation (b) (4)

3) Please submit a commitment to revise your drug substance lot release specifications to include quantitative data on the SDS-PAGE western blot analysis and IEF.

4) Please commit to performing stability studies showing that Zenapax manufactured by the improved GS-NSO process is stable for 4 hours at room temperature and for 24 hours at 2-8°C when diluted in 0.9% sodium chloride.

## APPENDIX I

### Comparability Studies

(b) (4)



2 Page(s) has been Withheld in Full as b4 (CCI/TS) immediately following this page

**APPENDIX II**

Viral Validation: vols. 13:60, 14:3

(b) (4)

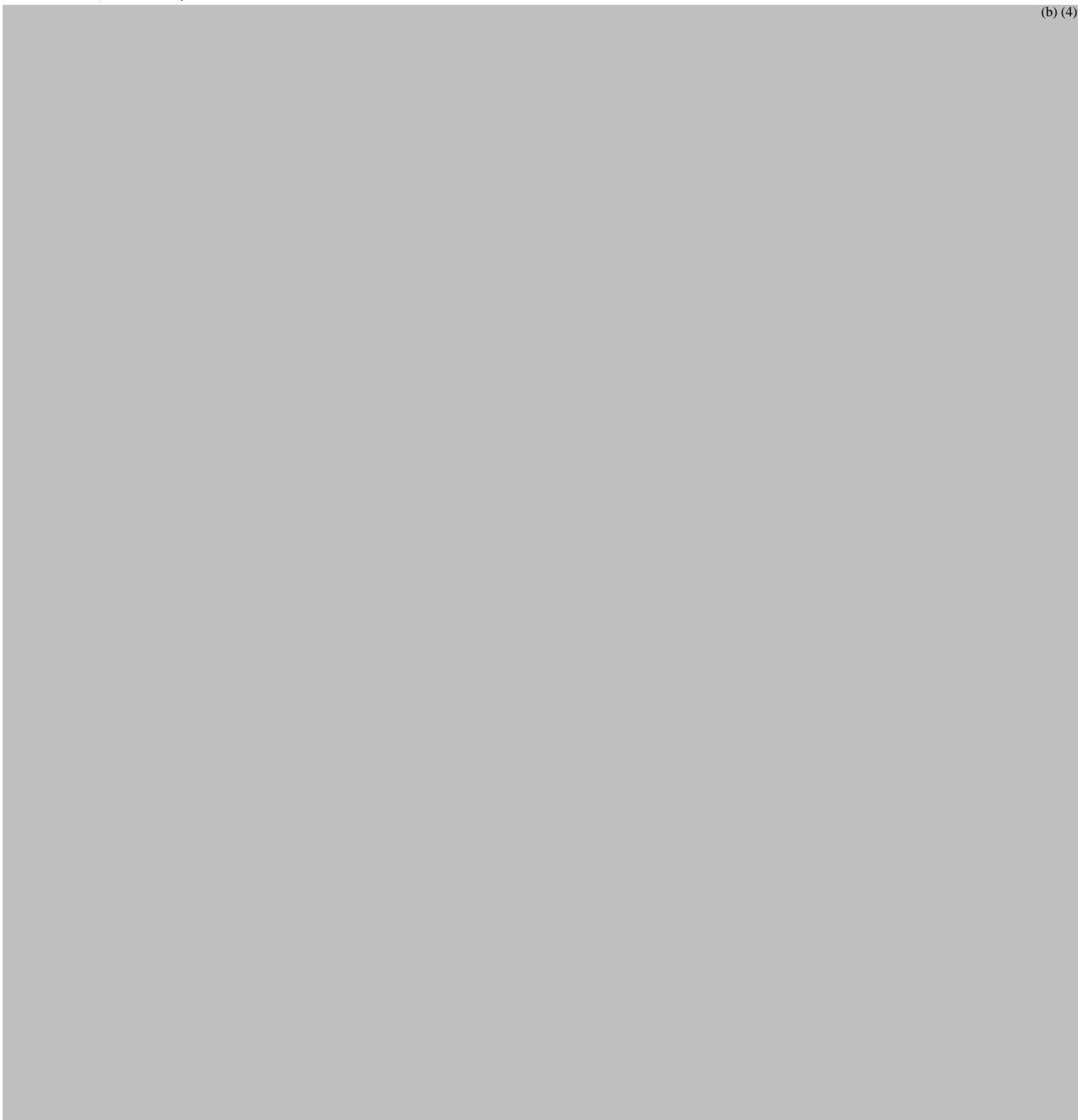


5 Page(s) has been Withheld in Full as b4 (CCI/TS) immediately following this page

**APPENDIX III**

**ASSAY VALIDATION**

**Show assay validation for release specs:**



(b) (4)