CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761029Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
BLA: 761029
Submission date: 2/27/15
Drug: daclizumab
Applicant: Biogen Idec Inc.
Indication: relapsing forms of multiple sclerosis

Reviewing Division: Division of Neurology Products

Discussion:
Toxicity studies of daclizumab were conducted in monkeys with up to 39 weeks duration. Skin toxicity was noted. Skin adverse events were also noted in humans. Microglial aggregates were also noted in the brain and spinal cord of monkeys. Initially, there was some uncertainty as to whether a no observed adverse effect level had been identified for this finding. The applicant provided some additional information and it appears that the low dose used in these studies (10 mg/kg) was the NOAEL for microglial aggregates. This dose produced exposures in monkeys approximately 7 times the exposure in humans at the recommended dose. The supervisor memo concluded that these findings of microglial aggregates do not preclude approval of daclizumab for several reasons. These reasons include the presence of a NOAEL and margin to human exposure, the minimal severity of the findings, and the lack of histopathology findings that might be sequelae to microglial activity.

An appropriate established pharmacologic class for daclizumab is the existing term, “interleukin-2 receptor blocking antibody.”

Conclusions:
The pharmacology/toxicology reviewer and supervisor conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that the nonclinical information is adequate to support approval for the above indication. I have provided labeling comments separately.

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/s/

PAUL C BROWN
05/24/2016

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: May 11, 2016

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: BLA 761029 (daclizumab High Yield Process; ZINBRYTA)

BLA 761029 was submitted by the sponsor (Biogen Idec) on February 27, 2015, to support marketing approval of daclizumab High Yield Process (DAC HYP, ZINBRYTA), an anti-CD25 humanized IgG1 monoclonal antibody, for treatment of relapsing forms of multiple sclerosis (RMS). Clinical development was conducted under IND 12120 for RMS. DAC HYP was also in clinical development for moderate to severe persistent asthma under IND (b) (4) which was terminated on March 3, 2016.

The nonclinical program consists of the following pivotal studies of DAC HYP:

- Pharmacology
- PK/ADME
- Toxicology
    - 13- and 39-week toxicity studies in cynomolgus monkey
    - 9-month toxicity study in cynomolgus monkey
- Reproductive and developmental toxicology
    - Fertility and early embryonic development studies in male and female cynomolgus monkey (separate studies)
    - Embryofetal development study in cynomolgus monkey
    - Pre- and postnatal development study in cynomolgus monkey

Nonclinical studies were reviewed in detail by Dr. Robison under IND (b) (4) (*cf. Pharmacology/Toxicology Review, IND* (b) (4) *Timothy W. Robison, Ph.D., March 4, 2008*) and Dr. Carbone under the BLA (*cf. Pharmacology/Toxicology BLA Review and Evaluation, BLA 761029, David L. Carbone, Ph.D., February 24, 2016*). Based on his review, Dr. Carbone has concluded that the nonclinical data do not support approval, based on toxicity observed in monkey (i.e., microglial aggregates in the CNS), which he considers serious and not monitorable in humans.

The following is a summary of the nonclinical data and selected safety issues; a comprehensive description and discussion of the data are provided in Dr. Carbone's review.

Pharmacology

DAC HYP (daclizumab) is a humanized IgG1 monoclonal antibody, which exhibits specific binding to CD25, the alpha subunit of the human high-affinity IL-2 receptor. The high affinity IL-2 receptor is "..rapidly upregulated after T-cell activation, resulting in enhanced high-affinity Il-2 signal transduction" (Wynn D *et al. Lancet Neurol* 9:381-390, 2010). DAC HYP is thought to be efficacious in the treatment of MS through binding to CD25 and subsequent inhibition of T-cell activation and proliferation (Milo R *Ther Adv Neurol Disord* 7(1):7-21, 2014; Wynn D *et al*., 2010).

No secondary or dedicated safety pharmacology studies were conducted.

PK/ADME

PK parameters were assessed in cynomolgus monkey in an acute-dose study of DAC HYP (5 mg/kg IV); the $t_{1/2}$ was ~8-16 days, Cl was 0.14-0.18 mL/hr/kg, and the Vd was 54-61 mL/kg. In acute and repeat dose studies in monkey, the bioavailability following SC dosing was 50-80%. In multiple-dose (Q2W) studies, accumulation was estimated to be ~2-fold, consistent with the reported $t_{1/2}$.

Studies in pregnant and lactating monkeys demonstrated placental transfer (umbilical cord blood:maternal blood ratios of 0.1-0.8) and excretion in milk (milk:serum ratios of 0.00028-0.0012 and 0.0005-0.00114 on PND 28 and 91, respectively). In infants, serum daclizumab levels were quantitated on PNDs 14, 28, and 91. The mean $t_{1/2}$ in infants was 15.3 ± 2.8 days. Maternal:infant serum exposure ratios were 1, 1.1, and 2.4 on PND 14, 28, and 91.

Toxicology

General toxicity studies were conducted in cynomolgus monkey, the only pharmacologically relevant species. The pivotal studies were 13- and 39-week studies and a 9-month study. All were conducted with DAC HYP, administered Q2W by subcutaneous injection, and included 3-month recovery periods. The doses were as follows:

- 13-week: 0, 5, 50, 125, and 200 mg/kg (recovery: 0, 125, and 200 mg/kg)
- 39-week: 0, 10, 50, and 200 mg/kg (recovery: 0 and 200 mg/kg)
- 9-month: 0, 10, 35, and 200 mg/kg (recovery: 0, 35, and 200 mg/kg)

The presence of anti-drug antibodies (ADA) was assessed in all the studies. While ADAs were detected, and associated with decreases in plasma exposure in some animals, the adequacy of the studies was not adversely affected.

The primary DAC HYP-related target organs were skin and CNS (brain and spinal cord).

Skin lesions were observed only in the 39-week and 9-month studies. In the 39-week study, clinical signs consisted of dry, red, and darkened skin, observed at all doses. The severity of the skin findings led to premature sacrifice of one LDF on Study Day 210. Histopathology correlates consisted of acanthosis/hyperkeratosis, subacute/chronic inflammation of the dermis, epidermal serocellular crust, and sebaceous gland atrophy, also observed at all doses. Similar skin lesions were observed in HD recovery animals. The sponsor concluded that there was no NOAEL for skin lesions.

The 9-month study was conducted to "…more definitively define an NOAEL in a chronic study…," although the same LD (10 mg/kg) was used in both studies. In the 9-month study, skin lesions were primarily observed at the MD and HD, and consisted of clinical signs of dry skin, gross findings of yellow scale, and histopathology findings of "…acanthosis, hyperkeratosis, and perivascular mononuclear inflammatory infiltrates." The sponsor identified the LD as an NOAEL; however, the final Pathology Report stated that "…animals dosed at all levels with Daclizumab showed a slightly increased incidence of minimal or mild chronic inflammatory changes consisting of acanthosis, hyperkeratosis, and perivascular mononuclear inflammatory infiltrates, compared to controls." The histopathology data are summarized in the following table.

FINDING	**DOSES (mg/kg)**							
	MALES				**FEMALES**			
	0	**10**	**35**	**200**	**0**	**10**	**35**	**200**
DAY 268 SKIN BIOPSY								
acanthosis								
minimal	1/4	2/4	1/4	1/4	1/4	0/4	1/4	2/4
mild	0/4	0/4	1/4	0/4	0/4	0/4	1/4	0/4
total	**1/4**	**2/4**	**2/4**	**1/4**	**1/4**	**0/4**	**2/4**	**2/4**
hyperkeratosis								
minimal	0/4	0/4	2/4	0/4	0/4	0/4	1/4	0/4
mild	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4
total	**0/4**	**0/4**	**2/4**	**0/4**	**0/4**	**0/4**	**2/4**	**0/4**
perivascular mononuclear cell infiltrate								
minimal	**1/4**	**3/4**	**2/4**	**1/4**	**0/4**	**1/4**	**4/4**	**2/4**
DAY 274 SACRIFICE								
acanthosis								
minimal	1/4	2/4	3/4	2/4	2/4	1/4	2/4	1/4
mild	0/4	0/4	0/4	0/4	0/4	0/4	1/4	2/4
total	**1/4**	**2/4**	**3/4**	**2/4**	**2/4**	**1/4**	**3/4**	**3/4**
hyperkeratosis								
minimal	**0/4**	**0/4**	**1/4**	**2/4**	**0/4**	**1/4**	**1/4**	**3/4**
perivascular mononuclear cell infiltrate								
minimal	1/4	1/4	1/4	3/4	2/4	1/4	3/4	2/4
mild	0/4	1/4	2/4	0/4	0/4	1/4	0/4	1/4
total	**1/4**	**2/4**	**3/4**	**3/4**	**2/4**	**2/4**	**3/4**	**3/4**

DAC HYP-induced skin lesions were also detected in humans. In her review of the clinical safety data (*cf. Clinical Review, BLA 761029, Maria Lourdes Villalba, M.D., March 23, 2016*), Dr. Villalba stated that "…40% of patients [had] "…at least one AE in

the skin and subcutaneous disorders…involving a great variety of reactions from mild reactions manageable with topical treatment with or without drug discontinuation to life-threatening reactions or death, despite drug discontinuation…Cutaneous events occurred at any time during the studies but more often after several months or years of treatment. Of the 894 patients with rash, 13% had a rash that was a SAE, was severe in intensity or led to drug withdrawal."

CNS lesions (i.e., microglial aggregates) were first observed in the male reproductive capacity study. In the original histopathology evaluation, microglial aggregates were detected "…in the brains of several treated animals." As a result, an expanded neurohistopathology evaluation was conducted in all animals, which included taking of 16 additional sections (left hemisphere, to include cortex, cerebrum, and cerebellum) and use of additional stains (GFAP, Fluoro-Jade B). The data are summarized in the following table.

BRAIN FINDING	DOSES (mg/kg)			
	0	10	50	200
MAIN STUDY				
mononuclear cell infiltrate				
choroid plexus; multifocal	0/5	0/5	2/5	1/5
meninges; multifocal	4/5	3/5	4/5	5/5
perivascular; focal	0/5	0/5	0/5	1/5
perivascular; multifocal	2/5	1/5	5/5	4/5
vacuolation; white matter; multifocal	1/5	2/5	2/5	1/5
hemosiderin; macrophage; perivascular; multifocal	5/5	4/5	5/5	4/5
microglial aggregates; multifocal	0/5	0/5	2/5	5/5
RECOVERY				
mononuclear cell infiltrate				
choroid plexus; multifocal	0/3			1/3
meninges; multifocal	0/3			3/3
perivascular; focal	3/3			1/3
perivascular; multifocal	0/3			0/3
vacuolation; white matter; multifocal	0/3			0/3
hemosiderin; macrophage; perivascular; multifocal	3/3			3/3
microglial aggregates; multifocal	0/3			0/3

Microglial aggregates were stated to be of minimal severity and to occur "…most frequently in the brainstem below the cerebellum, but were otherwise randomly scattered through the sections… in both gray and white matter…but more frequently in the white matter." In addition to brain, microglial aggregates were detected in "the section of thoracic spinal cord" in one MDM.

The peer review pathologist noted that "The Fluoro-Jade C stained slides were not of sufficient quality to make any diagnostic determinations." Therefore, the presence of degenerating neurons was not evaluated.

As a result of these findings, CNS tissue from the 13- and 39-week toxicity studies was re-examined and histopathology of brain was added to the female reproductive capacity

study. (No CNS findings were detected in the female reproductive capacity study at doses of 0, 10, 50, and 200 mg/kg Q2W for ~60 days.)
In addition, a Pathology Working Group (PWG) was convened to re-evaluate slides of brain and spinal cord from the 13- and 39-week studies. As stated in their report,

> "The PWG examined all sections of brain with a previous diagnosis recorded by either the study or reviewing pathologist. The PWG did not examine sections of brain that were diagnosed with no remarkable lesions by the study and reviewing pathologists. The PWG also examined all sections of spinal cord from all control and high-dose male and female monkeys from both the terminal and recovery sacrifice. All sections of spinal cord from Groups 2, 3 and 4 were examined by the PWG Chairperson confirming the absence of findings as reported by the study pathologist."

13-week toxicity study

The PWG findings in brain are summarized in the following table:

BRAIN FINDING	DOSES (mg/kg)									
	MALES					FEMALES				
	0	5	50	125	200	0	5	50	125	200
MAIN STUDY										
mononuclear cell infiltrate	0/3	1/3	0/3	2/3	3/3	1/3	1/3	2/3	3/3	2/3
microglial aggregates	0/3	0/3	0/3	2/3	2/3	0/3	0/3	1/3	2/3	1/3
RECOVERY										
mononuclear cell infiltrate	0/3			1/3	0/3	1/3			1/3	0/3
microglial aggregates	0/3			0/3	0/3	0/3			0/3	0/3

No DAC HYP-related findings were detected in spinal cord. The microglial aggregates in brain were characterized by the PWG as follows:

> Microglial aggregates consisted of focal accumulations of mononuclear cells within the parenchyma of the brain, most of which appeared to be microglial cells. The lesions did not appear to be active but consisted of a focal accumulation of mature appearing microglial cells within the neuropil. Microglial aggregates were observed in varying regions of the brain including the cerebral cortex, cerebellum, midbrain, and pons, without a predilection for a particular site. Although focal aggregates of microglial cells were present in a few animals, most were multifocal and all were considered to be of minimal severity. All foci were quite small and generally consisted of fewer than 30 cells.
>
> The random distribution of the microglial aggregates was inconsistent with a neurotoxic effect of the test article. Furthermore, there was no histologic evidence of neuronal degeneration, axonal fragmentation, or demyelination in association with the microglial aggregates. Therefore, it was the opinion of the PWG that the focal aggregates of microglial cells in the brain of monkeys in

the 50, 125 and 200 mg/kg dose groups were most likely secondary to a pharmacologic effect (immunomodulation) of the test article, presumably a host response to an unspecified infectious agent. The minimal severity and quiescent nature of the changes observed may explain the failure to observe a causative agent.

39-week toxicity study

The PWG findings are summarized in the following table:

TISSUE	FINDING	DOSES (mg/kg)							
		MALES				FEMALES			
		0	10	50	200	0	10	50	200
MAIN									
brain	mononuclear infiltrate	2/4	3/4	2/4	4/4*	2/4	2/4	1/4	4/4
	microglial aggregates	0/4	0/4	2/4	3/4	0/4	0/4	0/4	4/4
	microhemorrhage	0/4	0/4	0/4	1/4	0/4	0/4	0/4	1/4
spinal cord	mononuclear infiltrate	--	--	--	1/4	--	--	--	1/4
	microglial aggregates	--	--	--	2/4	0/4	0/4	0/4	1/4
RECOVERY									
brain	mononuclear infiltrate	1/3			2/3	1/3			2/3
	microglial aggregates	0/3			0/3	0/3			1/3
spinal cord	mononuclear infiltrate	--			0/3	--			0/3
	microglial aggregates	--			0/3	--			0/3

*in one HDM, the infiltrate in the choroid plexus was stated to be "…more extensive and of moderate severity." That in the other affected animals was characterized as "…generally minimal to slight in severity."

The microglial aggregates were characterized by the PWG exactly as in the PWG report for the 13-week study, except for the use of "single" to describe the focal aggregates in a few animals.

According to the Toxicology Summary, "A single microglial aggregate was found in one section of the brain of one animal dosed with 10 mg/kg/dose…" The affected animal could not be identified in the individual animal data, and, from the description of the PWG methodology, it appears unlikely that the PWG would have examined brain tissue from the affected animal.

The mononuclear infiltrate, characterized as perivascular (lymphohistiocytic) and focal/multifocal, was detected in the "parenchyma, meninges, and choroid plexus."

9-month toxicity study

Findings in brain and spinal cord identified by the study pathologist and the peer review pathologist are summarized in the following table.

TISSUE	FINDING	DOSES (mg/kg)							
		MALES				FEMALES			
		0	10	35	200	0	10	35	200
MAIN									
brain	mononuclear infiltrate	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
	microglial aggregates	0/4	1/4	0/4	3/4	0/4	0/4	2/4	3/4
	microhemorrhage	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
spinal cord	microglial aggregates	0/4	0/4	0/4	3/4	0/4	0/4	0/4	1/4
RECOVERY									
brain	mononuclear infiltrate	0/2		0/2	0/2	0/2		0/2	0/2
	microglial aggregates	0/2		1/2	0/2	0/2		1/2	0/2
spinal cord	microglial aggregates	0/2		1/2	0/2	0/2		0/2	0/2

Regarding the microglial aggregates detected in 1 LDM, the sponsor stated that "A single microglial aggregate was found in one section…, whereas more numerous microglial aggregates were generally observed in sections of the brain…" in MD and HD animals and concluded that the finding in the LDM "…could not be distinguished from an incidental change and was considered of equivocal relationship to the test article."

The sponsor also referenced historical control data from Charles River Laboratories (Butt MT et al. *Toxicol Path* 43:513-518, 2015). According to the publication, "…Biogen Idec contributed to the design, analysis, and interpretation of data, writing, reviewing, and approving the publication…," although the sponsor stated that data from the DAC HYP studies were not included. Of the toxicity studies conducted by the sponsor, only the 9-month toxicity study was conducted (b) (4); the 13- and 39-week studies were conducted (b) (4)

Butt et al. (2015) reported a background incidence of 7% (5/76) for microglial aggregates (i.e., "Focal gliosis consists of microglia" or "Focal gliosis comprises microglia with intermixed lymphocytes") in brain of control cynomolgus monkeys from 9 toxicology studies. The authors noted that the number of sections taken and examined ranged from 2 to 15 per animal and that "The probability of identifying microscopic change was unavoidably increased among animals having more slides examined…" From the information provided, it was not possible to correlate findings with the number of sections examined for any individual animal. A statement as to the number of slides of CNS tissue examined in the 39-week study could not be located in the study or the pathology reports. In the 9-month study, one H&E stained section of brain (no regions specified) and one of spinal cord were examined per animal for microscopic changes.

Considering potential differences in the methodologies used, the different testing facilities, the limited number of animals included in the database, and the fact that microglial aggregates were not detected in any control animal in the DAC HYP studies, the relevance of the historical control data to the DAC HYP studies is unclear. It is difficult to dismiss the microglial aggregates reported at the same LD in two separate studies. However, the sponsor's submission is inconsistent regarding the reporting of an affected LD animal in the 39-week study. The sponsor has been asked to address this issue (teleconference and email communication, May 5, 2016). If the sponsor confirms that there was an affected LD animal, then the LD should be considered an effect-dose.

Plasma exposures at the LD (10 mg/kg) were as follows:

STUDY	SAMPLING TIME	MALES		FEMALES	
		C_{max} (µg/mL)	$AUC_{(0\text{-}7d)}$ (mg*hr/mL)	C_{max} (µg/mL)	$AUC_{(0\text{-}7d)}$ (mg*hr/mL)
39-week	19th dose	113	28.2	168	45.9
9-month	Day 267	203	31.5	173	26.8

At the recommended human dose (RHD) of 150 mg Q4W, the plasma C_{max} and $AUC_{(0\text{-}28d)}$ were ~30 µg/mL and 630 µg*day/mL (or ~15 mg*hr/mL), respectively. The lowest mean plasma AUC at 10 mg/kg in monkey is ~7 times that at the RHD in humans (AUCs in monkey are multiplied by 4 to extrapolate from 0-7 days to 0-28 days).

Special Studies

A two-week neurotoxicity study was conducted, at the request of the division, to assess whether or not additional neurohistopathological changes may have been missed in the chronic studies, considering the late evaluation in those studies (*cf. Memorandum of Meeting Minutes, IND 12,120, dated August 22, 2008*). DAC HYP was administered to male cynomolgus monkeys at SC doses of 0, 10, 35, and 200 mg/kg either on Day 1 or on Days 1 and 15. Animals receiving a single dose were sacrificed either on Day 4 or Day 28/29 (recovery); those receiving two doses were sacrificed on Day 18 or Day 74 (recovery). Neurobehavioral assessments (general behavior, motor function, cranial nerve function [vision, movement of eyes, eyelid, response, mastication/jaw tone, facial expression, hearing and maintenance of head stability, breathing], and proprioception) were conducted at baseline, on Day 4 (all groups), Days 10 and 18 (all groups receiving two doses), and Day 28/29 (MD and HD recovery animals receiving one dose), and Day 74 (C, MD, and HD recovery animals receiving two doses). No adverse effects were observed. At necropsy, no gross pathology findings were detected. The histopathology evaluation included use of special stains (i.e., Luxol fast blue/cresyl violet, Fluoro-Jade B, GFAP, and immunohistochemistry [IBA-1, CD3, and CD20]) in selected main-study animals only. Microscopic findings in brain and spinal cord are summarized in the following table:

FINDINGS	MAIN STUDY					RECOVERY		
	DOSE	DOSES (mg/kg)				DOSES (mg/kg)		
		0	10	35	200	0	35	200
mixed cell infiltrates (brain, spinal cord, meninges)	D1	0/4	0/4	0/4	2/4		0/3	1/3
glial aggregates		0/4	0/4	0/4	3/4		0/3	1/3
hemorrhage (main study); hemosiderin (recovery)		0/4	0/4	0/4	2/4		0/3	0/3
glial aggregates (IBA-1 positive)		0/2			2/2			
parenchyma, increased IBA-1 staining		0/2			0/2			
mixed cell infiltrates (brain, spinal cord, meninges)	D1, D15	0/4	0/4	0/4	2/4	0/3	0/3	0/3
glial aggregates		0/4	0/4	0/4	3/4	0/3	0/3	1/3
hemorrhage (main study); hemosiderin (recovery)		0/4	0/4	0/4	1/4	0/3	0/3	1/3
glial aggregates (IBA-1 positive)					2/2			
parenchyma, increased IBA-1 staining					2/2			

Findings were characterized in the Pathology Report as follows:

- mixed cell infiltrates (brain and spinal cord)
    - "The infiltrates were mixtures of lymphocytes and neutrophils…" in the meninges and brain and spinal cord parenchyma.
    - The lymphocytic infiltrates were B- and T-cells, with T-cells "slightly" predominating in the meninges and B-cells "slightly" predominating in the parenchyma.
- Glial aggregates (brain and spinal cord)
    - "The aggregates were microglial cells (verified using the IBA-1 stain) in various regions of the brain and spinal cord."
    - They were "…generally graded as either slight or minimal and widely scattered throughout the brain and spinal cord, but seemed to be most numerous in the brain stem (medulla oblongata)."
    - They were "…predominantly, but not exclusively, in gray matter areas" and "…occasionally contained lymphocytes as confirmed by immunohistochemistry." Both B- and T-cells were detected in the aggregates.
- Hemorrhage (brain)
    - "Hemorrhage…was rare and present in two animals in the medulla oblongata. This finding was always a very slight component of the inflammatory reaction associated with the test article."

The study pathologist further noted that "The microglial aggregates and areas of mixed cell infiltrates were not associated with neuronal necrosis…" and were verified to be microglia, as they were "strongly IBA-1 positive…" In addition, "The cellular infiltrates and microglial aggregates were decidedly multifocal/scattered and not diffuse." Both were characterized as "nearly resolved" in recovery animals, although "Similar changes were still present in one of three high dose animals sacrificed on Day 28/29 after a 4-week recovery period following a single…200 mg/kg dose of the test article. Rare glial aggregates were observed in one of three high dose animals after an 8-week recovery period following …two…200 mg/kg doses of the test article." The study pathologist did not, however, comment on the relevance of these finding for human risk.

The sponsor conducted two <u>non-GLP in vitro studies</u> to test hypotheses (sponsor's description below) regarding the mechanism underlying the microglial aggregates observed in monkey with DAC HYP.

- Hypothesis 1: "Microglial cells express CD25 and DAC HYP has direct effects on microglia."
- Hypothesis 2: "Microglial cells do not express CD25 and DAC HYP has indirect effects on microglia as a consequence of changing IL-2 bioavailability within the CNS."

<u>Study R&D/13/953</u> was conducted in primary human fetal microglial cells. Using monoclonal antibodies that bind selectively to human CD25 and flow cytometric analysis, no binding was detected, indicating that the primary human fetal microglial cells

do not express CD25. However, "abundant" binding was demonstrated using antibodies specific for the subunits (CD122 and CD132) of the intermediate affinity form of the IL-2 receptor. Incubation of human fetal microglial cells with IL-2 resulted in microglial proliferation, which was not affected by blocking of CD25 by addition of DAC HYP antibody fragments to the culture.

Study R&D/13/970 was conducted in primary cynomolgus monkey microglial cells, using methods similar to those used in Study R&D/13/953. The data demonstrated that cynomolgus monkey microglial cells express functional intermediate IL-2 receptors but not CD25. As with primary human fetal microglial cells, IL-2 stimulated microglial proliferation, which was not affected by DAC HYP antibody fragments.

From the results of these studies, the sponsor concluded that the microglial aggregates observed in monkey are not a direct effect of DAC HYP. According to the sponsor,

> "Because cynomolgus monkey microglial cells express functional intermediate IL-2 receptors but not CD25, we hypothesize a likely mechanism responsible for the aggregation of microglial cells into foci change in microglial motility within the CNS of DAC HYP treated cynomolgus monkeys is related to DAC HYP's IL-2 modulating effects when CD25 expressing cells are saturated within the microenvironment of the CNS…"

The sponsor reported that IL-2 is elevated (2-3 fold) in serum and in csf (*personal communication from Dr Bibiana Bielekova of the National Institutes of Neurological Diseases and Stroke*) of DAC HYP-treated MS patients. The sponsor provided no data on the effects of DAC HYP on IL-2 levels in cynomolgus monkey. The sponsor also provided no data from in vitro studies of adult human microglial cells.

Assuming that the data from human fetal microglial cells are applicable to adult human microglial cells, the data overall suggest that the microglial aggregates may be an indirect effect of DAC HYP-induced increases in CNS levels of IL-2. However, this would not mitigate concerns regarding risk to humans if IL-2 is, in fact, elevated in humans. While the sponsor proposes a protective role for the microglial aggregates (i.e., "…regulating IL-2 within their microenvironment through consumption of IL-2, thereby decreasing the IL-2 levels and making IL-2 unavailable for the stimulation of antigen reactive effector T cells within the CNS compartment…"), the only "evidence" provided by the sponsor is that in none of the monkey studies were microglial aggregates associated with neuronal degenerative or necrotic changes.

Reproductive and Developmental Toxicology

The reproductive and developmental toxicity of DAC HYP was tested in the following studies in cynomolgus monkey: reproductive capacity in males and females (separate studies), embryofetal development, and pre- and postnatal development.

Because fertility studies in monkey are not feasible, potential effects on fertility were assessed indirectly. In males, sperm parameters, serum testosterone, and histopathology of reproductive organs, brain, and spinal cord were evaluated in sexually mature animals (5-10.5 years). DAC HYP was administered by SC injection at doses of 0, 10, 50, and 100 mg/kg Q2W for ~60 days. Animals were sacrificed 1 week after the last dose (all groups) or after a 12-week recovery period (C and HD only). An expanded neurohistopathology evaluation was conducted following detection of microglial aggregates using standard methodology. In females, menstrual cycle, serum estradiol and progesterone, and histopathology of female reproductive organs, adrenal and pituitary glands, and brain were evaluated. DAC HYP was administered by SC injection at doses of 0, 10, 50, and 200 mg/kg Q2W for ~60 days. Animals were sacrificed 35-37 days after the last dose (all groups) or after a 60-day recovery period (C and HD only).

No DAC HYP-related effects on fertility parameters were observed in either males or females. The only clearly DAC HYP-related finding was histopathological changes in brain (discussed in the Toxicology section) and injection site reactions ("minimal to mild perivascular mononuclear cell infiltrates…in the subcutis…in a few animals administered 50 or 200 mg/kg…") in males.

In the embryofetal development study, DAC HYP was administered to pregnant females at SC doses of 0, 10, 50, and 200 mg/kg QW on GDs 20-50. The only DAC HYP-related developmental finding was an increase in fetal loss at the HD (1, 1, 1, and 3 fetuses at C, LD, MD, and HD, respectively). Histopathology was not evaluated in adults or fetuses.

In the pre- and postnatal development study, DAC HYP was administered to pregnant females at SC doses of 0 and 50 mg/kg QW on GDs 50-160. Offspring were sacrificed on PND 180-181. A full necropsy was conducted on all infants; however, histopathology was conducted only on gross lesions, brain (cerebrum, midbrain, medulla/pons, and cerebellum), and spinal cord (cervical, thoracic, and lumbar). Terminal procedures were not conducted on adults. No DAC HYP-related findings were observed in the offspring.

Expert Opinion: The sponsor provided assessments (b) (4) addressing the fetal loss observed in the embryofetal development study. Both individuals concluded that the fetal loss was unrelated to drug, for the following reasons:

- Lighter weigh animals were assigned to the HD group, and animals weighing less have a greater tendency for fetal loss (b) (4)
- "Single fetal losses prior to GD 50 occurred in the control and low dose groups, demonstrating the tendency of monkeys to experience pregnancy loss" (b) (4) .
- "No additional developmental toxicity endpoints…were observed" and "In the pre-postnatal toxicity study of daclizumab, no increase in abortions or total fetal deaths was observed over the period from early gestation to 6 months of age" (b) (4)

None of these arguments are sufficiently compelling to warrant dismissal of a DAC HYP-related increase in fetal loss in the embryofetal development study. While it is correct that a decrease in offspring survival was not observed in the pre- and postnatal study, the single dose level in that study was 50 mg/kg, whereas the dose associated with fetal loss in the embryofetal development study was 200 mg/kg. Therefore, the increase in fetal loss is considered DAC HYP-related and should be described in labeling.

Genetic Toxicology

Genetic toxicology studies were not conducted because, as a monoclonal antibody, DAC HYP is not expected to interact directly with DNA.

Carcinogenicity

Because DAC HYP is pharmacologically active only in monkey, carcinogenicity studies are not feasible; therefore, the carcinogenic potential of DAC HYP was not assessed.

Conclusions and Recommendations

The nonclinical studies conducted to support clinical development and marketing approval for DAC HYP for treatment of patients with relapsing forms of MS were adequate by design and conduct. The primary toxicities observed in the one relevant species, cynomolgus monkey, were skin lesions and microscopic findings in brain and spinal cord. The skin lesions were detected at clinically relevant plasma DAC exposures and have been observed in humans, with humans appearing more sensitive than monkey.

The primary finding in brain and spinal cord was the presence of microglial aggregates. Microglial aggregates were detected "…throughout the gray and white matter of the brain (including but not limited to the cerebral cortex, cerebellum, thalamus, hippocampus, midbrain, and pons) and the spinal cord" in a dose-related manner. Microglial aggregates, always report to be of minimal severity, were multifocal (a single focus identified in one or two animals was considered a spontaneous finding) and were, in some animals, associated with evidence of microhemorrhage and inflammation. However, in no case were they characterized as active or associated with evidence of neuronal degeneration or necrosis. The PWG, which reviewed sections of brain and spinal cord from the male reproductive study and the 13- and 39-week toxicity studies, concluded that the microglial aggregates appeared inactive and their widespread distribution throughout the brain and in spinal cord was not consistent with a direct neurotoxic effect. The sponsor's mechanistic studies were inadequate to identify a cause, although based on the results the sponsor suggested that the microglial aggregates may have been the result of DAC HYP-induced increases in IL-2, with subsequent activation of intermediate affinity IL-2 receptors. The sponsor reported increases in circulating IL-2 in humans but not monkey. Therefore, if this is the mechanism underlying the microglial aggregates detected in monkey, the sponsor has provided data demonstrating the human relevance of this toxicity.

Microglia are “recognized as the prime components of an intrinsic brain immune system” and are involved in both acute and chronic neuroinflammation (Streit WJ *et al. Journal of Neuroinflammation* 1(1) (2004): 14). As a contributor to chronic inflammation, microglial activation is thought to contribute to the pathology underlying neurodegenerative diseases, including multiple sclerosis (Streit WJ *et al.,* 2004). Therefore, Dr. Carbone’s concern regarding the potential for DAC HYP-induced increases in microglial aggregates in MS patients is reasonable, particularly, as he notes, an increase in microglial aggregates would not be monitorable in humans. However, there are several observations that reduce that concern: (1) the severity of the finding was always characterized as minimal, even at the highest doses tested and for the longest duration of dosing, (2) while associated with evidence of microhemorrhage and inflammation, microglial aggregates were never found to be associated with “neuronal degeneration, axonal fragmentation, or demyelination…,” even in the focused neurohistopathology study requested by the division, (3) in the tissue slides, from multiple toxicity studies, examined by the PWG, the microglial aggregates “did not appear to be active” and were found to be widespread throughout the brain, which is not characteristic of a direct neurotoxic insult, and (4) although not definitively identified as a NOAEL, the LD (10 mg/kg), associated with a plasma AUC ~7 times that in humans at the RHD, was associated with only a single microglial aggregate in one animal in each of the chronic toxicity studies (if confirmed for the 39-week study, as requested by the division).

Overall, the nonclinical studies conducted by the sponsor are adequate to support marketing approval of DAC HYP, with appropriate labeling. The primary organ toxicities identified were skin lesions, which have already been detected in humans, and CNS lesions (microglial aggregates), which cannot be monitored in humans. However, for the reasons stated, it is my opinion that the microglial aggregates are not of sufficient concern to preclude approval, particularly if the clinical team concludes that there is sufficient evidence of efficacy in humans to warrant approval in light of the serious toxicities already demonstrated in humans.

No PMRs are recommended. The general requirement for a juvenile animal toxicology study is waived because the clinical team has concluded that the serious toxicities observed in adults administered DAC HYP preclude its use in the pediatric population.

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/s/

LOIS M FREED
05/11/2016

Study Title: Embryo-Fetal Development of Daclizumab Administered by Subcutaneous Injection to Pregnant Cynomolgus Monkeys.

Study no. TR07123

This replaces the table of fetal measurements on page 44 of the Pharmacology/Toxicology BLA Review and Evaluation of daclizumab (BLA 761029).

Dose (mg/kg)	**0**	**5**	**50**	**200**
Fetal Measurements				
Sex:				
male	10	5	5	2
female	2	7	6	10
Crown-to-Rump	--	--	↓7%	↓5%
Crown-to-Heel	--	--	--	↓4%
Foot Length	--	↓3%	↓8%	↓3%
Body Weight	--	--	↓11%	↓11%

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/s/

DAVID L CARBONE
03/24/2016

LOIS M FREED
03/24/2016

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number:	BLA 761029
Supporting document/s:	1
Applicant's letter date:	February 27, 2015
CDER stamp date:	February 27, 2015
Product:	ZINBRYTA™ (Daclizumab high yield process; DAC-HYP)
Indication:	Relapsing forms of multiple sclerosis
Applicant:	Biogen Idec Inc.
Review Division:	Division of Neurology Products
Reviewer:	David L Carbone, Ph.D.
Supervisor:	Lois M Freed, Ph.D.
Division Director:	Billy Dunn, M.D.
Project Manager:	Laurie Kelly, PA-C

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 761029 are owned by Biogen Idec Inc. or are data for which Biogen Idec Inc. has obtained a written right of reference.
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1 Executive Summary

1.1 Introduction

Daclizumab-High Yield Process (DAC-HYP; ZINBRYTA™) is a humanized monoclonal IgG1 antibody developed by Biogen Idec Inc. for the treatment of relapsing forms of multiple sclerosis. DAC-HYP is thought to suppress T-cell mediated autoimmunity by selectively targeting the CD25 subunit of the high-affinity IL-2 receptor, while leaving the intermediate-affinity (non-CD25) receptors functional.

1.2 Brief Discussion of Nonclinical Findings

In vitro studies demonstrated suppression of high-affinity IL-2 receptors by DAC-HYP in primary lymphocyte cultures isolated from humans and monkeys. There was no pharmacological activity in primary lymphocytes isolated from rodents. All *in vivo* toxicology studies were, therefore, conducted in cynomolgus monkeys.

Biweekly subcutaneous (SC) dosing with up to 200 mg/kg DAC-HYP for 5 dosing periods did not affect sperm morphology in males, or hormonal or menstrual cycles in females. Once-weekly SC dosing with 50 mg/kg DAC-HYP during gestation days (GD) 50-160 did not affect pre- or postnatal development. However, once-weekly SC administration of 200 mg/kg DAC-HYP administered during the first 5 weeks of pregnancy increased the incidence of fetal loss. A juvenile animal toxicology study was not required prior to filing the BLA. However, if DAC-HYP is approved for use in adolescent patients, a juvenile animal toxicology study is recommended.

No toxicity was associated with single intravenous (IV) doses up to 30 mg/kg. Pivotal toxicology was initially evaluated with a 39-week study in males and females administered 0, 10, 50, or 200 mg/kg DAC-HYP by biweekly SC injection. A second study of similar design but of 9-months duration was conducted to evaluate doses of 0, 10, 35, or 200 mg/kg. The primary toxicities in the repeat-dose studies were raised, red/patchy areas of the skin and scattered microglial aggregates throughout the brain and spinal cord. Dermal and CNS toxicities generally resolved over recovery periods ranging from 4 to 12 weeks. Dermal toxicity was accompanied microscopically by acanthosis and was typically only observed after repeat dosing. There was no NOAEL for dermal toxicity. The microglial aggregates were occasionally accompanied by mononuclear cell infiltration or signs of hemorrhage (i.e., hemosiderin deposits), but no signs of axonal degeneration or myelin loss were observed. An acute dose study revealed the presence of microglial aggregates after a single dose of 200 mg/kg SC, but there were no accompanying behavioral abnormalities. In the 39-week and 9-month studies, the NOAEL for microglial aggregates was 10 mg/kg administered biweekly ($AUC_{(0\text{-}7d)} \cong 34{,}988$ μg×h/mL).

The sponsor has proposed that the dermal and CNS toxicities in monkeys are due to increases in plasma IL-2 levels that result in stimulation of intermediate-affinity IL-2 receptors. In support, the sponsor cites increases in plasma IL-2 levels in humans treated with DAC-HYP; however, plasma IL-2 was not measured in any of the nonclinical studies. Two *in vitro* studies were conducted in an attempt to identify a

mechanism responsible for the microglial aggregates. These studies demonstrated proliferation of human and monkey primary microglial cells in response to IL-2 stimulation in the absence of CD25 at the IL-2 receptor, suggesting that activation of the microglial cells by IL-2 occurred through intermediate-affinity IL-2 receptors. A DAC-HYP fragment which lacks the Fc region did not suppress IL-2 mediated microglial activation, further implicating the intermediate affinity IL-2 receptor in microglial activation. Studies evaluating a direct interaction between DAC-HYP and microglial cells were not conducted.

The sponsor's proposed mechanism for the dermal and CNS toxicities is plausible but remains unproven without more definitive studies. Moreover, the biological significance of the microglial aggregates remains unclear.

1.3 Recommendations

1.3.1 Approvability

The dermal toxicity observed in nonclinical testing has been observed in clinical trials. However, it is unknown if microglial aggregates are similarly replicated in humans, since this finding cannot be monitored in a clinical setting. Concern over the microglial aggregates is heightened due to the unknown effect of this finding on any existing neuroinflammation in the intended patient population. Based on steady state exposures in monkeys at the NOAEL and humans receiving the proposed clinical dose, the safety margin for the microglial aggregates is approximately 9-fold. Given an inadequate safety margin for the microglial aggregates and the availability of alternate therapy for relapsing forms of MS, DAC-HYP is not recommended for approval.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

8.1 (b) (4)

Risk Summary

(b) (4)

(b) (4)

Animal Data

(b) (4)

8.2 (b) (4)

~~8.2 Lactation~~

Risk Summary

(b) (4)

(b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

The carcinogenic potential of ZINBRYTA has not been (b) (4)

(b) (4)

(b) (4)

Mutagenesis

(b) (4)

Impairment of Fertility

(b) (4)

13.2 Animal Toxicology and/or Pharmacology

(b) (4)

There was a dose-dependent increase (b) (4) in (b) (4) microglial aggregates in the brain and spinal cord of monkeys at doses greater than 10 mg/kg (b) (4)

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): 152923-56-3

Generic Name: Daclizumab-High Yield Process (DAC-HYP)

Code Name: BIIB019

Molecular Formula/Molecular Weight: 144 kDa

Structure or Biochemical Description: DAC-HYP is a recombinant, humanized monoclonal immunoglobulin gamma-1 (IgG1) antibody, purified from an NS0 cell line. The general structure is indicated in the figure below.

(Sponsor's figure)

Pharmacologic Class: Interleukin-2 receptor blocking antibody.

2.2 Relevant INDs, NDAs, BLAs, and DMFs

DAC-HYP was previously developed under IND (b) (4) for the treatment of severe, persistent asthma, but development was discontinued. DAC-HYP was developed under IND 012120 for the treatment of relapsing forms of MS.

2.3 Drug Formulation

DAC-HYP for injection is formulated at 150 mg/mL in (b) (4) mM succinate (b) (4), (b) (4) mM sodium chloride, and (b) (4) % Tween 80.

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

DAC-HYP is intended to be used in (b) (4) adult patients with relapsing MS. The proposed dose of 150 mg is to be administered once monthly by subcutaneous injection, using prefilled syringes.

2.7 Regulatory Background

IND 012120 was submitted January 1, 2005. A pre-BLA meeting between the FDA and AbbVie was held on October 8, 2014; there were no nonclinical questions except

related to abuse potential. There were no nonclinical filing issues for the BLA; a decision to file was reached on April 6, 2015. Ownership of DAC-HYP was transferred from AbbVie to Biogen Idec, Inc. on May 12, 2015. On August 13, 2015, the sponsor submitted a waiver request for the juvenile animal toxicology study, and on November 2, 2015, a juvenile animal toxicology study protocol was submitted.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

- R&D/14/0566: Cross-reactivity study of DAC HYP binding to murine and rat peripheral blood mononuclear cells.
- RTR31: Characterization of DAC-HYP in human lymphocyte functional assays in vitro.
- RTR4: BIAcore-based affinity measurements of daclizumab HYP (b) (4) and Daclizumab Nutley to recombinant human and cynomolgus CD25.
- RTR41: Comparison of anti-CD25 humanized monoclonal antibodies DAC-HYP and non-FcR-binding DAC (b) (4) in human lymphocyte functional assays in vitro.
- RTR56: Characterization of DAC-HYP ADCC and FcγRIII (CD16) interactions in vitro.
- RTR57: A comparison of DAC-HYP, Roche Nutley, and Roche Penzburg daclizumab materials in in vitro human lymphocyte functions.
- TR05312: Daclizumab manufacturing lot characterization; Comparison of daclizumab materials in antibody-dependent cell cytotoxicity and complement-dependent cytotoxicity assays.
- TR05332: Physiochemical/biological characterization of comparative stability testing of daclizumab high yield process engineering lots compared to Roche current process lots.
- TR05333: Physiochemical and biological characterization of daclizumab HYP (b) (4) bulk lots, 41110401, 41110403, 41110405.

Pharmacokinetics

- DAC.08.03: Validation of an ELISA Method for Quantification of Daclizumab (DAC-HYP) in Monkey Serum.
- CST019-17VR: DAC study, Validation of an Enzyme Linked Immunosorbent Assay (ELISA) Method for Quantification of Daclizumab in Cynomolgus Monkey Serum.
- TR00212.1: Qualification of Daclizumab Cynomolgus Serum PK ELISA.

- TR01112: DAC study, Validation of an Enzyme Linked Immunosorbent Assay (ELISA) Method for Quantification of Daclizumab in Cynomolgus Monkey Serum.
- TR03147: Partial Validation of Quantification of Daclizumab in Cynomolgus Serum by ELISA.
- TR04127: Partial Validation of an ELISA for Quantitation of Daclizumab in Cynomolgus Serum.
- TR04196: Partial Validation of an ELISA for Detection and Quantitation of Antibodies to Daclizumab in Cynomolgus Serum by ELISA.
- TR04222: Daclizumab, Partial Validation for Relocated Methods, Partial Validation of a Cell-based Flow Cytometry Ligand Binding Assay for Detection of Neutralizing Antibodies to Daclizumab in Cynomolgus Monkey and Human Serum.
- TST019-023VR and TST019-023VR-R.1: Validation of an Enzyme Linked Immunosorbent Assay (ELISA) Method for Quantification of Daclizumab in Cynomolgus Monkey Milk.
- TR11337: Partial Validation of an ELISA for the Quantitation of Daclizumab in Cynomolgus Serum and Long Term Stability of Daclizumab in Matrix.
- CST019-018VR: Validation of an ECL Method for Detection of Anti-Daclizumab Antibodies in Monkey Serum.
- TR03174: Validation of a Cell-based Flow Cytometry Ligand Binding Assay for Detection of Neutralizing Antibodies to Daclizumab in Cynomolgus Monkey and Human Serum.
- TR04217: Daclizumab Final Report Pharmacokinetics and Comparability Analyses for PDL Study PDL.Daclizumab-04.001, Pharmacokinetic Comparison of Three Manufacture Lots of Daclizumab Administered as a Single IV Bolus in Cynomolgus Monkeys.

Single Dose Toxicology

- TR07133: A single dose intravenous acute toxicity study of daclizumab in cynomolgus monkeys.

Repeat-Dose Toxicology

- TR04236: A repeat dose (once every two weeks for three doses) tolerability and toxicokinetic study of daclizumab administered by subcutaneous injection to cynomolgus monkeys, with a 2-month recovery period.
- TR05395_1: 13-week subcutaneous injection toxicity and toxicokinetic study with daclizumab in cynomolgus monkeys with a 12-week recovery period.
- TR07185_3: A 39-week subcutaneous injection chronic toxicity and toxicokinetic study with daclizumab in cynomolgus monkeys with a 12-week recovery period.

- P019-11-01: A 9-month chronic toxicity study of daclizumab administered once every 2 weeks by subcutaneous injection to cynomolgus monkeys with a 12-week recovery period.
- P019-08-01: A 2-week subcutaneous acute toxicity study in male cynomolgus monkeys with a 4-week or 8-week recovery.

Reproductive and Developmental Toxicology

- TR07135_2: A Male Reproductive Toxicology Study of Daclizumab Administered Bi-Weekly by Subcutaneous Injection to Cynomolgus Monkeys, with a Recovery Period.
- TR06121-1: A Female Reproductive Toxicology Study of Daclizumab Administered Bi-Weekly by Subcutaneous Injection to Cynomolgus Monkeys, with a Recovery Period.

Embryonic and Fetal Development

- TR07122: Preliminary Embryo-Fetal Development Study of Daclizumab Administered by Subcutaneous Injection to Pregnant Cynomolgus Monkeys.
- TR07123: Embryo-Fetal Development Study of Daclizumab Administered by Subcutaneous Injection to Pregnant Cynomolgus Monkeys.

Prenatal and Postnatal Development

- TC11-033: Pre-Postnatal Toxicity of Daclizumab HYP (b) (4) Administered by Subcutaneous Injection to Pregnant Cynomolgus Monkeys with a 6-Month Postnatal Evaluation.

Special Toxicology Studies

- R&D-13-953: Characterization of Interleukin 2 (IL-2) receptor protein expression and IL-2 proliferation responses by primary human microglial cells.
- R&D-13-970: Characterization of Interleukin 2 (IL-2) receptor protein expression and IL-2 proliferation responses by primary cynomolgus monkey microglial cells.
- TR06098: Local Tolerance Study in Rabbits (Intravenous Injection) with Daclizumab.
- TR06080: Hemolysis assay in human whole blood with daclizumab.
- TR08279: Method Qualification Study to Establish the Conditions for Cross-Reactivity of Zenepax and Daclizumab HYP with normal Human and Cynomolgus Monkey Tissues.
- TR08278: Cross-Reactivity Study of Daclizumab HYP with Normal Human and Cynomolgus Monkey Tissues.

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Summaries are provided for the nonclinical studies reviewed under IND (b) (4) by Timothy Robison, Ph.D. (review dated 3/4/2008).

4 Pharmacology

4.1 Primary Pharmacology

DAC-HYP is thought to suppress T-cell mediated autoimmunity in relapsing MS by targeting the CD25 subunit of the high-affinity IL-2 receptor, while not affecting the intermediate (non-CD25) IL-2 receptor pathway. *In vitro* studies demonstrated that DAC-HYP prevents IL-2 induced proliferation and cytokine secretion in human and monkey primary lymphocytes. DAC-HYP had no pharmacological effect on primary lymphocytes isolated from rats or mice. Plasmon surface resonance revealed comparable affinity of DAC-HYP for human (K_D = 0.49 nM) and monkey (K_D = 0.42 nM) CD25. DAC-HYP and a mutant antibody which is unable to bind Fcγ receptors (FcγR) were used to distinguish CD25-dependent and independent pharmacologic activities in human lymphocyte cultures. Both antibodies suppressed IL-2 induced proliferation. However, the mutant antibody did not down-regulate CD25 expression, nor induce antibody-dependent cell-mediated cytotoxicity (ADCC), suggesting that its effects on IL-2 are CD25 independent. An *in vitro* receptor binding panel suggested that DAC-HYP-mediated ADCC likely occurred through interaction with the FcγIIIR. DAC-HYP did not induce complement-dependent cytotoxicity.

4.2 Secondary Pharmacology

None

4.3 Safety Pharmacology

None

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

A standard battery of PK/ADME studies was not conducted with DAC-HYP. A single IV bolus of 5 mg/kg DAC-HYP in male and female cynomolgus monkeys (6/sex) revealed a plasma half-life of 136-305 h. ELISA methods were developed and validated for measuring DAC-HYP in cynomolgus monkey serum and milk. An ECL method using biotinylated DAC was developed and validated for measuring anti-DAC-HYP antibodies in monkey serum. A flow cytometry method was established and validated for measuring DAC-HYP-neutralizing antibodies in monkey serum.

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: A single dose intravenous acute toxicity study of daclizumab in cynomolgus monkeys.

Study no.:	TR07133
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	4/25/06
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DAC-HYP, lot # 90670404, 99.3%

Methods

Doses:	0, 15, 30 mg/kg
Frequency of dosing:	Single dose followed by 15-day recovery.
Route of administration:	IV infusion (2-3 min)
Dose volume:	1.5-3.0 mL/kg
Formulation/Vehicle:	150 mg DAC-HYP in (b) (4) mM succinate (b) (4), (b) (4) mM sodium chloride, and (b) (4) % Tween 80.
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	1/sex/group
Age:	3.1-5.9 years
Weight:	Males = 3.2-4.5 kg; Females = 2.5-3.3 kg.
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	No significant deviations.

Summary of Findings

(Based on review of IND (b) (4) by T. Robison, Ph.D., 3/4/2008)

The objective of this study was to evaluate the acute toxicity associated with DAC-HYP administration in monkeys. No adverse clinical signs were observed, and there were no differences in food consumption or body weight between the drug and control groups. Increases in C_{max} and AUC_{last} were dose-proportional. The plasma half-life ranged from 8 to 16 days. Blood samples were analyzed using standard clinical chemistry and hematology panels on study Days 3 or 15; there were no abnormal findings. Following recovery, a full necropsy was performed on all animals, but descriptions of histological findings were limited to the injection site. No adverse effects were noted; the NOAEL was defined as 30 mg/kg.

6.2 Repeat-Dose Toxicity

Study title: A repeat dose (once every two weeks for three doses) tolerability and toxicokinetic study of daclizumab administered by subcutaneous injection to cynomolgus monkeys, with a 2-month recovery period.

Study no.:	TR04236
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	7/9/04
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	Daclizumab-HYP Lot #I06259A, 98.4%

Methods

Doses:	5, 50, 125, 200 mg/kg.
Frequency of dosing:	QD, study Days 1, 15, and 29.
Route of administration:	SC injection
Dose volume:	2 mL/kg
Formulation/Vehicle:	(b) (4) mg/mL DAC-HYP in (b) (4) mM sodium succinate, (b) (4) mM sodium chloride with (b) (4) % Tween 80, pH 6.0.
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	3/sex/group
Age:	3.3-4.1 yrs (males); 2.9-3.5 yrs (females)
Weight:	2.6-3.3 kg (males); 2.2-2.9 kg (females)
Satellite groups:	None
Unique study design:	This study consisted only of clinical observations, hematology, clinical chemistry, and PK analysis. All animals were monitored throughout the recovery period, and were returned to the animal colony upon completion of the study.
Deviation from study protocol:	No significant deviations.

Observations and Results

Mortality

All animals survived the study and were returned to the animal colony on study Day 91.

Clinical Signs

Cageside evaluations were performed twice daily; there were no drug-related clinical signs.

Body Weights

There were no differences in weight gain among the study groups.

Food Consumption

Food consumption was evaluated qualitatively. Reduced food consumption was reported in two females in the 125 mg/kg group but did not correlate with any reductions in weight gain.

Hematology

Hematology and clinical chemistry were evaluated on study Days 3, 17, and 31. There were no remarkable hematology findings.

Clinical Chemistry

Clinical chemistry revealed elevations over baseline in ALT, AST, and LDH in 1/3 females (50 mg/kg) and 3/3 males (200 mg/kg) within 48 h of the first dose (e.g., Day 3). These findings subsequently resolved and were not observed after the second or third doses.

Dose (mg/kg)	Sex	Animal No.	Day	ALT	AST	LDH
50	F	24108	3	↑6.2×	↑18×	↑6.2×
200	M	2440	3	↑3.2×	↑4.7×	↑3.4×
	M	2448	3	↑3.4×	↑5.5×	↑4.2×
	M	2453	3	↑1.6×	↑2.2×	↑5.4×

(Study Day 3: Liver function tests)

Toxicokinetics

TK analyses were conducted after the 1st and 3rd doses. Increases in C_{max} and AUC were generally dose-proportional. At all doses, C_{max} was reached approximately 48 h after dosing, and plasma half-life ranged from 9 to 13 days. Decreases in serum DAC-HYP were observed in 1/3 LD females (see below; Animal No. MF24103), but were attributed to the anti-daclizumab antibodies detected in this animal.

Table 1. Main TK Parameters for All Dose Groups

Dose	Group	Animal #	Gender	C_{max} (µg/mL)		AUC_τ (hr*mg/mL)		AUC_{ss}	t_{max} (day)		$t_{1/2}$	R
(mg/kg)				First dose	Last dose	First dose	Last dose	(hr*mg/ml)	First dose	Last dose	(day)	$(AUC\tau)_{last\ dose} \div (AUC\tau)_{first\ dose}$
		MF2438M*	Male	44.45	80.01	12.33	19.58	21.13	3	3	10.35	1.588
		MF2445M*	Male	40.38	74.32	11.08	16.60	17.52	2	2	9.032	1.498
		MF2454M	Male	43.08	80.06	11.44	19.98	22.96	3	2	13.56	1.746
5	1	MF24103F*	Female[a]	44.65	BQL	10.55	NC	NC	2	NC	NC	NC
		MF24107F	Female	41.01	83.24	12.42	23.01	26.60	4	2	13.36	1.853
		MF2423F*	Female	47.67	90.58	13.28	20.85	22.50	3	2	10.40	1.570
		MF2439M	Male	531.4	952.7	130.0	217.4	243.2	4	2	12.12	1.673
		MF2447M	Male	552.7	1019	142.4	255.6	300.1	4	4	14.41	1.795
		MF2451M	Male	509.1	897.7	124.0	211.9	239.3	4	2	12.53	1.709
50	2	MF24108F	Female	535.9	843.9	125.6	216.7	247.1	2	2	13.06	1.725
		MF24114F	Female	571.6	998.2	144.0	244.8	273.6	2	2	11.93	1.699
		MF24122F	Female	548.7	938.1	126.3	218.3	251.0	2	1	13.52	1.729
		MF2415M	Male	1015	1625	247.9	367.7	395.3	3	2	9.077	1.483
		MF2437M	Male	1152	1896	281.8	427.5	473.7	2	2	9.960	1.517
		MF2442M	Male	1179	1695	262.3	337.3	351.7	2	2	6.547	1.286
125	3	MF24105F	Female	1152	2125	302.8	448.1	492.9	2	2	9.537	1.480
		MF24112F	Female	1144	1758	277.4	426.1	464.6	2	2	9.974	1.536
		MF2481F	Female	1509	2258	355.1	566.9	631.2	3	1	11.22	1.596
		MF2440M	Male	1524	3382	423.7	678.4	737.9	3	2	10.10	1.601
		MF2448M	Male	2084	3497	493.2	835.5	940.6	2	2	12.03	1.694
		MF2453M	Male	1935	3168	429.8	660.7	677.9	2	1	9.023	1.537
200	4	MF2424F	Female	1708	3338	456.0	727.9	793.9	3	2	10.34	1.589
		MF2477F	Female	2162	2651	384.2	490.0	483.1	2	1	5.747	1.275
		MF2479F	Female	1790	2899	426.6	650.2	700.6	1	3	9.696	1.524

*: Animals had detectable anti-DAC antibodies after DAC treatment [3]
[a]: This animal had measurable drug level up to Day 8, only limited parameters were estimated.
NC: Not computed

(Sponsor's Table)

Dosing Solution Analysis

Preformulated DAC-HYP was provided by the sponsor. Dosing solutions were within ±5% of the target concentrations.

Study title: 13-week subcutaneous injection toxicity and toxicokinetic study with daclizumab in cynomolgus monkeys with a 12-week recovery period.

Study no.:	TR05395_1
Study report location:	EDR
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	11/16/2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DAC-HYP, Lot 90670401 (96.8%) and 90670402 (96.7%).

Methods

Doses:	0, 5, 50, 125, 200 mg/kg (main); 0, 125, 200 mg/kg (recovery)
Frequency of dosing:	QD every 2 weeks (total of 7 doses over 13 weeks)
Route of administration:	SC injection
Dose volume:	2 mL/kg
Formulation/Vehicle:	(b) (4) mg/mL DAC-HYP in (b) (4) mM sodium succinate, (b) (4) mM sodium chloride with (b) (4) % Tween 80, pH 6.0.
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	3/sex/group (main); 3/sex/group (recovery)
Age:	2-2.5 years
Weight:	1.5-2.1 kg (males); 1.5-2.3 kg (females)
Satellite groups:	None
Unique study design:	A pathology working group was convened to evaluate microglial aggregates.
Deviation from study protocol:	No significant deviations

Summary of Findings

(Based on review of IND (b) (4) by T. Robison, Ph.D., 3/4/2008)

All animals survived until scheduled necropsy. There were no drug-related effects on body weight gain or food consumption, nor were there any adverse ophthalmologic or ECG effects. Clinical chemistry and urinalysis did not reveal any abnormal findings. Hematology parameters were generally unremarkable. There were no abnormal gross pathology or organ weights findings. Histological evaluation revealed focal and multifocal microglial aggregates in males (≥125 mg/kg) and females (≥50 mg/kg). Focal microglial aggregates were also observed in the spinal cord of 1 female (200 mg/kg). No microglial aggregates were found following the recovery period. The microglial aggregates appeared to be randomly distributed and were minimal in severity. No evidence of neuronal degeneration, axonal fragmentation, or demyelination was noted, and the aggregates appeared to be partially resolved at the end of the recovery period. A mechanism responsible for the microglial aggregates was not determined.

Tissue/Finding	Sex	Main Study (mg/kg)					Recovery (mg/kg)		
		0	5	50	125	200	0	125	200
Brain									
Infiltrate, mononuclear cell	M	0/3	1/3	0/3	2/3	3/3	1/3	1/3	0/3
	F	1/3	1/3	2/3	3/3	2/3	1/3	1/3	0/3
Microglial aggregate, focal/multifocal, minimal	M	0/3	0/3	0/3	2/3	2/3	0/3	0/3	0/3
	F	0/3	0/3	1/3	2/3	1/3	0/3	0/3	0/3
Spinal Cord									
Microglial aggregate, focal/multifocal, minimal	M	0/3	0/3	0/3	0/3	0/3	1/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3

Serum samples for TK analysis were collected on Days 1, 2, 4, 8, 15, 29, 71, 73, 76, 79, 85, 87, 99, 113, 141, and 170. Serum samples for anti-daclizumab antibody assessments were collected on study Days 1 and 85 from all animals and Day 177 from the recovery group. Exposure increased in a dose-dependent manner. Loss of DAC-HYP exposure due to daclizumab-neutralizing antibodies occurred by Day 29 in 3 monkeys at 5 mg/kg and 1 monkey at 125 mg/kg. By Day 99, 1 monkey from the 200 mg/kg group also lost exposure due to neutralizing antibodies. Based on the microglial findings, the NOAEL for this study was 5 mg/kg (C_{max} = 62.8 μg/mL, AUCτ = 15,208 h×μg/mL).

Dose (mg/kg	Sex	$t_{1/2}$ (h)	1st Dose			6th Dose		
			t_{max} (h)	C_{max} (μg/mL)	AUC_τ (h×μg/mL)	t_{max} (h)	C_{max} (μg/mL)	AUC_τ (h×μg/mL)
5	M	NA	56.1	44.7	10717	49.2	59.6	13777
	F	NA	71.9	40.3	10633	121.8	69.3	18072
50	M	NA	40.2	408.5	102457	49.3	658.4	161731
	F	NA	56	398.1	100460	49.3	583.7	133150
125	M	274.1	55.7	1016.6	256033	49.3	1554.2	362053
	F	258.9	63.8	960.7	240219	49.4	1727.2	402522
200	M	267.8	55.8	1700.1	426637	49.5	2363.0	551481
	F	328.4	71.8	1565.6	370986	73.6	2414.8	599139

Study title: A 39-week subcutaneous injection chronic toxicity and toxicokinetic study with daclizumab in cynomolgus monkeys with a 12-week recovery period.

Study no.: TR07185_3
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 5/3/2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Daclizumab-HYP, lot #90670405, 97.7%

Methods

Doses:	0, 10, 50, 200 mg/kg (main); 0, 200 mg/kg (recovery)
Frequency of dosing:	QD, every two weeks
Route of administration:	SC injection
Dose volume:	2 mL/kg
Formulation/Vehicle:	(b) (4) mg/mL in (b) (4) mM sodium succinate, (b) (4) mM sodium chloride, and (b) (4) % Tween 80
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	4/sex/group (main); 3/sex/group (recovery)
Age:	1.7-3.3 years
Weight:	1.7-2.4 kg (males); 1.7-2.0 kg (females)
Satellite groups:	None
Unique study design:	A pathology working group was convened to evaluate microglial aggregates in the brain and spinal cord.
Deviation from study protocol:	No significant deviations

Summary of Findings

(Based on review of IND (b) (4) by T. Robison, Ph.D., 3/4/2008)

All animals were observed twice daily for mortality or morbidity. One LDF (I04868) was euthanized on study Day 210 due to unresolving dermal toxicity. Skin lesions in this animal were first observed on Day 169 and consisted of dry skin around the body extremities and orifices. Raised, plaque-like lesions were observed on the face, with possible vesicles on the eyelids, chin, and cheeks. Upon necropsy, large, bilateral lymph nodes were observed. Histologic examination revealed marked acanthosis/hyperkeratinosis, moderate subacute to chronic inflammation, and marked sebaceous gland atrophy.

Clinical signs in the remaining animals included a dose-responsive increase in the frequency of broken, dry, or red skin in males and females, which did not completely resolve during the recovery period in the HD animals.

Parameter	Males				Females			
	0	10	50	200	0	10	50	200
Animals/group	7	4	4	7	7	4	4	7
Qualitative low food consumption	3	1	0	6	4	2	2	6
Broken skin								
Broken skin, mouth	0	0	0	2	0	0	0	0
Broken skin, left front digit(s)	0	0	0	0	0	0	0	1
Dry skin								
Dry skin, arms	0	0	1	1	0	2	0	1
Dry skin, entire head	0	0	0	1	0	0	1	0
Dry skin, hind legs	0	0	0	1	0	2	1	2
Dry skin, midline inguinal	0	1	1	1	1	2	2	2
Dry skin, midline, ventral abdomen	0	1	0	2	1	1	1	2
Dry skin, mouth	0	1	0	3	0	1	1	2
Dry skin, muzzle	0	0	0	1	0	0	0	0
Dry skin, nose	0	0	0	1	0	0	0	0
Dry skin, midline ventral thorax (chest)	0	1	0	0	0	0	0	1
Dry skin, all legs	0	0	0	0	1	0	2	1
Red skin								
Red skin, arms	0	1	0	4	0	2	0	2
Red skin, entire head	0	0	0	1	0	0	1	0
Red skin, hind legs	0	0	0	2	1	1	0	0
Red skin, left arm	0	0	0	2	0	1	0	1
Red skin, left hind leg	0	0	0	1	0	1	0	0
Red skin, left mandible	0	0	0	1	0	0	0	0
Red skin, left ventral abdomen	0	0	0	1	0	0	0	0
Red skin, lips	0	0	0	1	1	0	0	1
Red skin, mouth	0	0	0	1	0	1	1	1
Red skin, muzzle	0	0	0	2	0	0	0	0
Red skin, right arm	0	0	0	1	0	1	0	0
Red skin, scrotum	0	0	0	1	-	-	-	-
Red skin, midline ventral abdomen	0	1	0	2	1	1	1	2
Red skin, midline ventral thorax (chest)	0	3	0	1	0	0	0	1
Red skin, right lateral thorax (chest)	0	0	0	0	0	0	0	1
Darkened skin								
Darkened skin, all legs	0	0	0	1	0	0	0	0
Darkened skin, arms	0	0	0	1	0	0	0	0
Darkened skin, hind legs	0	0	0	1	0	0	0	0
Darkened skin, mouth	0	0	0	2	0	0	0	0
Darkened skin, muzzle	0	0	0	1	0	0	0	0
Excretion								
Discolored feces, red in color	1	1	0	1	0	0	1	2

(Table from nonclinical review of IND (b) (4))

Dose-dependent decreases in body weight gain were observed in the males during the dosing period. Reduced weight gain in HD males persisted into the recovery period, while weight gain in females increased. Qualitative evaluation indicated decreases in food consumption in males and females during the dosing period.

Parameter	Males				Females			
	0	10	50	200	0	10	50	200
BW (kg), Wk1	2.0	2.0	2.1	2.1	1.9	1.8	1.8	1.9
BW (kg), Wk39	3.0	2.8	2.9	2.7	2.5	2.2	2.4	2.4
BW (kg), RWk12	3.4	-	-	3.0	2.8	-	-	2.8
ΔWk39-Wk1	1.0	0.8	0.8	0.6	0.6	0.4	0.6	0.5
% Initial BW (Wk1)	50.0	40.0	38.1	28.6	31.6	22.2	33.3	26.3
% Control BW gain	100	80	76.2	57.1	100	70.4	105.6	83.3
ΔRWk12-Wk39	0.4	-	-	0.3	0.3	-	-	0.4
%Initial BW (Wk39)	13.3	-	-	11.1	12.0	-	-	16.7
% Control BW gain	100.0	-	-	83.3	100.0	-	-	138.9

(Table from nonclinical review of IND (b) (4) *)*

There were no abnormal ophthalmoscopy or ECG observations. There were no drug-related effects on clinical chemistry parameters. Urinalysis revealed decreases in volume (43-63% of control) in the HD males. There were no drug-related effects on clinical chemistry. Hematology and peripheral blood immunophenotyping were generally unremarkable.

Gross pathology included drug-related red/patchy skin accompanied by histological signs of acanthosis/hyperkeratosis and subacute/chronic inflammation of the dermis.

Microglial aggregates in the brain and spinal cord were evaluated by a pathology working group. Focal and multifocal aggregates in the parenchyma of the brain consisted primarily of microglial cells. Aggregates were found in the cerebral cortex, cerebellum, midbrain, and pons, but there was no evidence of localization to any particular region. All foci consisted of ≤30 cells. No evidence of neuronal degeneration, axonal fragmentation, or demyelination was associated with the microglial aggregates. Minimal to slight focal and multifocal mononuclear infiltrates were found in the brain parenchyma, meninges, and choroid plexus. A mechanism responsible for the microglial aggregates was not determined by the working group. Following the recovery period, microglial aggregates were only detected in 1 HD female, suggesting that these effects are reversible. Evaluation of the spinal cord revealed small foci of microglial aggregates in 2 HD males and 1 HD female. No microglial aggregates were found in the spinal cord after the recovery period.

Tissue/Finding	Sex	Main Study (mg/kg)				Recovery (mg/kg)	
		0	10	50	200	0	200
Brain							
Infiltrate, mononuclear, perivascular, minimal to moderate, focal/multifocal	M	2/4	3/4	2/4	4/4	1/3	2/3
	F	2/4	2/4	2/4	4/4	1/3	2/3
Microglial aggregate, minimal, focal/multifocal	M	0/4	0/4	2/4	3/4	0/3	0/3
	F	0/4	0/4	0/4	4/4	0/3	1/3
Spinal Cord							
Infiltrate, mononuclear, perivascular, cervical	M	0/4	0/4	0/4	1/4	0/3	0/3
	F	0/4	0/4	0/4	0/4	0/3	0/3
Microglial aggregates, cervical	M	0/4	0/4	0/4	2/4	0/3	0/3
	F	0/4	0/4	0/4	0/4	0/3	0/3
Microglial aggregates, thoracic	M	0/4	0/4	0/4	0/4	0/3	0/3
	F	0/4	0/4	0/4	1/4	0/3	0/3

TK parameters were calculated after Doses 1, 10, and 19. Exposure increased in a dose-proportional manner. Elevations in C_{max} and AUC_{0-7d} suggesting accumulation of daclizumab following repeat dosing until steady state levels were achieved. Daclizumab-neutralizing antibodies were detected in 12 control animals, 2 LD animals, and 1 MD animal, resulting in decreased daclizumab exposure in the treated animals. The presence of daclizumab-neutralizing antibodies in the control group is concerning but may indicate non-specificity in the analytical method. Based on the microglial findings, the NOAEL for this study was 10 mg/kg (C_{max} = 141 μg/mL, AUC_{0-7d} = 37.1 h×mg/mL).

Dose (mg/kg)	Males				Females			
	T_{max} (h)	C_{max} (μg/mL)	AUC_{0-7d} (mg×h/mL)	$t_{1/2}$ (days)	T_{max} (h)	C_{max} (μg/mL)	AUC_{0-7d} (mg×h/mL)	$t_{1/2}$ (days)
Dose #1								
10	60.1	94.7	22.4	--	54.4	108	23.2	--
50	60.2	493	112	--	54.3	638	130	--
200	55.2	2274	466	--	58.6	2195	470	--
Dose #10								
10	59.9	153	37.4	--	59.7	185	46.8	--
50	53.9	800	195	--	41.7	893	213	--
200	47.9	2922	645	--	47.6	3486	815	--
Dose #19								
10	59.8	113	28.2	6.69	47.5	168	45.9	14.0
50	59.7	738	181	11.5	39.2	808	200	12.5
200	57.9	3050	656	6.69	43.8	3435	716	11.0

Study title: Daclizumab: A 9-month chronic toxicity study of daclizumab administered once every 2 weeks by subcutaneous injection to cynomolgus monkeys with a 12-week recovery period.

Study no.:	P019-11-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 25, 2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Daclizumab, Lot #VVJJ20, 100%

Methods

Doses:	0, 10, 35, 200 mg/kg
Frequency of dosing:	Once every two weeks, for 9 months, followed by a 12 week recovery period
Route of administration:	SC
Dose volume:	1.33 mL/kg
Formulation/Vehicle:	150 mg DAC-HYP in (b) (4) mM succinate (b) (4), (b) (4) mM sodium chloride, and (b) (4) % Tween 80
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	Main study, 4/sex/group
Age:	2.8-4.1 years
Weight:	Males, 2.5-3.0 kg; Females, 2.3-3.1 kg
Satellite groups:	Recovery group: 0, 35, 200 mg/kg, 2/sex/group
Unique study design:	A pathology working group was convened to evaluate the microglial aggregates in the brain and spinal cord.
Deviation from study protocol:	There were no significant deviations.

Observations and Results

Mortality

All animals were checked twice daily for mortality and morbidity; all animals survived until scheduled necropsy.

Clinical Signs

Cageside observations were performed once daily. Detailed clinical observations were performed once weekly for the duration of the study. Dry skin was noted in all groups but was more frequent in the MD and HD animals.

Dose	0 mg/kg/dose	10 mg/kg/dose	35 mg/kg/dose	200 mg/kg/dose
Number of Observations	34	8	258	423
Number of Affected Animals	3 of 8	3 of 8	7 of 8	8 of 8
Duration of Observations[1]	196-274	196-234	49-274	42-274

[1] Days from-to

(Sponsor's Table; Dermal toxicity)

Body Weights

Animals were weighed weekly for the duration of the study. Daily weight gain in the males was slightly lower in MD (2.2%) and HD (2.2%) groups compared to controls (3.2%). There were no drug-effects on weight gain in the females.

Food Consumption

Food consumption was qualitatively evaluated every morning for the duration of the study. Sporadic occurrences of low food consumption were noted but were not likely drug-related.

Ophthalmoscopy

Slit lamp and indirect ophthalmoscopy were performed once during the prestudy phase and once during week 39 of the main study. There were no adverse findings. No examinations were performed on the recovery animals.

ECG

Examinations were performed twice during the prestudy phase, on Study Day 129 and Week 39, and on the recovery group animals. There were no ECG abnormalities.

Hematology, Coagulation, Clinical Chemistry, and Urinalysis

Blood samples for hematology, coagulation, clinical chemistry, and urinalysis were collected according to the schedule below; there were no abnormal findings.

Samples for Clinical Pathology Evaluation

Group No(s).	Time Point[a]	Hematology	Coagulation	Clinical Chemistry	Urinalysis
All animals	Prestudy Day -14	X	N/A	X	N/A
All animals	Prestudy Day -7	X	X	X	X
All animals	Day 85[b]	X	N/A	X	X
All animals	Day 183[b]	X	N/A	X	X
All animals	Day 267[b]	X	X	X	X
Groups 1, 3, and 4,	Day 357	X	X	X	X
Unscheduled euthanasia (when possible)	Before euthanasia	X	X	X	_

X = sample to be collected; N/A = not applicable.
[a] Prestudy samples for hematology and clinical chemistry will be collected with a 7-10 day separation between the two sets of collections.
[b] Prior to dose

(Sponsor's Table)

Gross Pathology

DAC-HYP increased the incidence of acanthosis, which was characterized by yellow scale and thickening of the skin. Acanthosis persisted throughout the recovery period in 1/2 MD females, 1/2 HD males, and 1/2 HD females.

Finding	Sex	Main Study (mg/kg)				Recovery (mg/kg)		
		0	10	35	200	0	35	200
Skin								
Yellow scale	M	0/4	1/4	2/4	1/4	0/2	0/2	1/2
	F	2/4	0/4	1/4	3/4	0/2	1/2	1/2

Organ Weights

There were no drug-effects on organ weights.

Histopathology

Adequate Battery: Yes

Text Table 13
Tissue Collection and Preservation

Administration site	Large intestine, cecum
Animal identification	Large intestine, colon
Artery, aorta	Liver
Biopsy (skin; Week 39 and 50)	Lung
Bone marrow smear	Lymph node, axillary
Bone marrow, femur	Lymph node, mandibular
Bone marrow, sternum	Lymph node, mesenteric
Bone, femoral-tibial joint	Muscle, skeletal psoas, and diaphragm
Bone, femur	Nerve, optic[a]
Bone, sternum	Nerve, sciatic
Brain	Ovary
Cervix	Pancreas
Epididymis	Skin, biopsy
Esophagus	Skin
Eye[a]	Small intestine, duodenum
Gallbladder	Small intestine, ileum
Gland, adrenal	Small intestine, jejunum
Gland, mammary	Spinal cord
Gland, parathyroid	Spleen
Gland, pituitary	Stomach
Gland, prostate	Testis[b]
Gland, salivary mandibular	Thymus
Gland, seminal vesicle	Trachea
Gland, thyroid	Urinary bladder
Gross lesions/masses	Uterus
Gut-associated lymphoid tissue	Vagina
Heart	
Kidney	

[a] Preserved in Davidson's fixative.
[b] Preserved in Modified Davidson's fixative.

(Sponsor's Table)

Peer Review: Yes

Signed Pathology Report: Yes

Histological Findings:

Microglial Aggregates
Focal and multifocal CNS microglial infiltrates were observed at all doses. Aggregates were randomly scattered throughout the gray and white matter of the brain and were present in the cerebral cortex, midbrain, thalamus, hippocampus, basal ganglia, piriform complex, pons, medulla, and cerebellum. In one HD female, the microglial aggregates were accompanied by signs of minimal hemorrhage. Microglial aggregates were also observed in the spinal cord of 3 HD males and 1 HD female.

Finding	Sex	Main Study (mg/kg)				Recovery (mg/kg)		
		0	10	35	200	0	35	200
Brain								
Microglial aggregate	M	0/4	1/4	0/4	3/4	0/2	1/2	0/2
	F	0/4	0/4	2/4	3/4	0/2	1/2	0/2
Aggregate/mixed cells	M	0/4	0/4	0/4	0/4	0/2	0/2	0/2
	F	0/4	0/4	0/4	1/4	0/2	0/2	0/2
Hemorrhage	M	0/4	0/4	0/4	0/4	0/2	0/2	0/2
	F	0/4	0/4	0/4	1/4	0/2	0/2	0/2
Spinal Cord								
Microglial aggregate	M	0/4	0/4	0/4	3/4	0/4	0/4	0/4
	F	0/4	0/4	0/4	1/4	0/4	0/4	0/4

Dermal

Routine skin sections and punch biopsies revealed minimal or mild inflammatory changes that were dose-related. Dermal pathology did not completely resolve by the end of the recovery period.

Finding	Sex	Main Study (mg/kg)				Recovery (mg/kg)		
		0	10	35	200	0	35	200
Skin Section								
Acanthosis, minimal	M	1/4	2/4	3/4	2/4	0/2	2/2	0/2
	F	2/4	1/4	2/4	1/4	0/2	0/2	0/2
Acanthosis, mild	M	0/4	0/4	0/4	0/4	0/2	0/2	0/2
	F	0/4	0/4	1/4	2/4	0/2	0/2	1/2
Hyperkeratosis, minimal	M	0/4	0/4	1/4	3/4	0/2	0/2	0/2
	F	0/4	1/4	1/4	3/4	0/2	0/2	1/2
Mononuclear cell infiltrate, minimal	M	1/4	1/4	1/4	3/4	1/2	2/2	0/2
	F	2/4	0/4	3/4	2/4	0/2	0/2	0/2
Mononuclear cell infiltrate, mild	M	0/4	1/4	2/4	0/4	0/2	0/2	0/2
	F	0/4	1/4	0/4	1/4	0/2	0/2	1/2
Punch Biopsy								
Acanthosis, minimal	M	1/4	2/4	1/4	1/4	0/2	0/2	0/2
	F	1/4	0/4	1/4	2/4	0/2	1/2	0/2
Acanthosis, mild	M	0/4	0/4	1/4	2/4	0/2	0/2	1/2
	F	0/4	0/4	0/4	0/4	0/2	0/2	0/2
Hyperkeratosis, minimal	M	0/4	0/4	2/4	0/4	0/2	0/2	1/2
	F	0/4	0/4	1/4	0/4	0/2	0/2	0/2
Hyperkeratosis, mild	M	0/4	0/4	0/4	0/4	2/2	1/2	0/2
	F	0/4	0/4	1/4	0/4	0/2	1/2	0/2
Mononuclear cell infiltrate, minimal	M	1/4	3/4	2/4	1/4	0/2	0/2	1/2
	F	0/4	1/4	1/4	2/4	0/2	0/2	0/2

Peripheral Lymph Nodes

Minimal to moderate increased size or number of germinal centers of the axillary lymph nodes occurred in a dose-responsive pattern, suggesting an immune response to daclizumab-HYP. These findings did not completely resolve by the end of the recovery period.

Finding	Sex	Main Study (mg/kg)				Recovery (mg/kg)		
		0	10	35	200	0	35	200
Axillary lymph node								
Increased size/number, germinal center								
-Minimal	M	0/4	1/4	1/4	1/4	0/2	0/2	0/2
	F	0/4	1/4	0/4	1/4	0/2	0/2	1/2
-Mild	M	0/4	0/4	2/4	1/4	0/2	0/2	2/2
	F	0/4	2/4	3/4	2/4	0/2	1/2	1/2
-Moderate	M	1/4	0/4	0/4	0/4	0/2	1/2	1/2
	F	0/4	0/4	0/4	0/4	0/2	1/2	1/2
Paracortical expansion								
-Mild	M	0/4	0/4	0/4	0/4	0/2	1/2	0/2
	F	0/4	0/4	0/4	1/4	1/2	2/2	1/2
Mandibular lymph node								
Increased size/number, germinal center								
-Minimal	M	0/4	1/4	0/4	0/4	2/2	0/2	0/2
	F	1/4	0/4	0/4	0/4	0/2	0/2	0/2
-Mild	M	1/4	0/4	3/4	3/4	0/2	1/2	2/2
	F	0/4	2/4	1/4	3/4	2/2	1/2	1/2
-Moderate	M	0/4	0/4	0/4	1/4	0/2	0/2	0/2
	F	0/4	0/4	2/4	0/4	0/2	0/2	0/2
Paracortical expansion								
-Minimal	M	0/4	0/4	0/4	0/4	0/2	0/2	0/2
	F	0/4	1/4	0/4	0/4	0/2	1/2	0/2
-Mild	M	0/4	0/4	0/4	0/4	0/2	0/2	0/2
	F	0/4	0/4	1/4	0/4	0/2	0/2	0/2
Extramedullary hematopoesis								
-Minimal	M	0/4	0/4	0/4	0/4	0/2	0/2	0/2
	F	1/4	0/4	0/4	0/4	0/2	0/2	0/2
Mesenteric lymph node								
Increased size/number, germinal center								
-Minimal	M	1/4	0/4	1/4	0/4	0/2	0/2	0/2
	F	1/4	1/4	0/4	1/4	0/2	0/2	0/2
-Mild	M	1/4	0/4	2/4	1/4	0/2	0/2	0/2
	F	0/4	0/4	2/4	1/4	0/2	0/2	1/2
-Moderate	M	0/4	0/4	0/4	0/4	0/2	0/2	1/2
	F	0/4	0/4	1/4	0/4	0/2	0/2	0/2

Special Evaluation

Neutralizing Antibodies:
Blood samples for evaluating anti-daclizumab antibodies (ADA) were collected once during the predose phase, prior to dosing on study Days 127, 267, and 273, and on recovery Day 357. There was a low incidence of ADA, which did not affect the analysis or interpretation of the TK data.

Immunophenotyping:
Blood was collected on Days -14 and -7 of the predose phase, and prior to dosing on study Days 85, 183, and 267, and recover Day 357; immunophenotyping was performed by flow cytometry. Daclizumab did not induce changes in the number of B-lymphocytes, T-lymphocytes, T-helper and T-cytotoxic lymphocytes, natural killer (NK) cells, T-regulatory (Treg) lymphocytes, or Th17 lymphocytes. There were no changes in NK cell activity or T-cell proliferation. Elevations in IgG levels were observed at 200 mg/kg, although it was suggested by the sponsor that the assay used to quantify IgG was actually detecting DAC-HYP.

Toxicokinetics

Blood samples for TK analysis were collected on Days 1, 127, and 267. Increases in exposure were generally dose proportional. There were no sex differences. Based on the microglial findings, the NOAEL for this study was 10 mg/kg (C_{max} = 188 μg/mL, $AUC_{0\text{-}7d}$ = 29.2 h×mg/mL).

Dose (mg/kg)	Sex	Day 1			Day 127			Day 267		
		C_{max} (μg/mL)	T_{max} (h)	$AUC_{0\text{-}7d}$ (mg×h/mL)	C_{max} (μg/mL)	T_{max} (h)	$AUC_{0\text{-}7d}$ (mg ×h/mL)	C_{max} (μg/mL)	T_{max} (h)	$AUC_{0\text{-}7d}$ (mg×h/mL)
10	M	106	96	15.5	243	60	37.4	203	48	31.5
	F	98.9	96	14.2	178	60	25.9	173	72	26.8
35	M	390	72	56.100	745	64	116	725	48	108
	F	392	64	55.3	692	32	104	669	48	101
200	M	2290	72	299	3960	64	578	3150	32	460
	F	2090	72	286	4110	48	594	4220	64	624

Dosing Solution Analysis

All dosing solutions were within 5% of their respective target concentration.

Study title: DAC HYP: A 2-week subcutaneous acute toxicity study in male cynomolgus monkeys with a 4-week or 8-week recovery.

Study no.:	P019-08-01
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	10/20/2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DAC-HYP, Lot #90670504, 98.9%

Methods

Doses:	0, 10, 35, 200 mg/kg
Frequency of dosing:	QD on Day 1 (Groups 1-4) or Days 1 and 15 (Groups 5-8); see below.
Route of administration:	SC
Dose volume:	2 mL/kg
Formulation/Vehicle:	150 mg daclizumab-HYP in (b) (4) mM succinate (b) (4), (b) (4) mM sodium chloride, and (b) (4) % Tween 80
Species/Strain:	Male cynomolgus monkey
Number/Sex/Group:	4/group; 3/recovery group (0, 35, 200 mg/kg)
Age:	Described as “Young adult”
Weight:	2.5-4 kg
Satellite groups:	None
Unique study design:	Neurobehavioral assessments, and expanded neurohistopathology evaluation of the brains were included.
Deviation from study protocol:	No significant deviations

Experimental Design

Group Number	Number of Males		Test Materials	Dosage Level (mg/kg)	Dose Concentration (mg/mL)	Dosage Volume (mL/kg)	Dosing Regimen	Necropsy Day
	Main Study	Recovery						
1	4	0	Vehicle Control	0	0	2	SC once on Day 1	Main Study: Day 4 Recovery: Day 28/29[a]
2	4	0	DAC HYP	10	5			
3	4	3		35	17.5			
4	4	3		200	100			
5	4	3	Vehicle Control	0	0	2	SC once on Days 1 and 15	Main Study: Day 18 Recovery: Day 74
6	4	0	DAC HYP	10	5			
7	4	3		35	17.5			
8	4	3		200	100			

(Sponsor's Table)

Observations and Results

Mortality

All animals were checked twice daily for mortality or morbidity; all animals survived until scheduled necropsy.

Clinical Signs

Clinical observations were recorded at least twice daily. Isolated occurrences of dry/flaky skin, soft feces, erythema, and alopecia were observed sporadically across all groups.

Body Weights

Body weights were recorded on study Day -1, and once per week thereafter; there were no drug effects on body weight gain.

Food Consumption

Food consumption was qualitatively measured daily. There were no drug effects on food consumption.

Ophthalmoscopy

Ophthalmic evaluations were performed before initiation of dosing, and on Days 3 and 17; there were no drug-related findings.

ECG

Not evaluated

Hematology

The schedule for blood sample collection is described in detail below. On study Day 4, total lymphocytes in Groups 2 and 3 were decreased by 37% and 30%, respectively, but resolved over the recovery period. There were no drug effects on coagulation.

Blood Sample Collection Schedule

Time Point	Hematology	Coagulation	Serum Chemistry	ADAb	TK
Day -14 (All animals: Groups 1–8)	X	NA	X	NA	NA
Day -7 (All animals: Groups 1–8)	X	X	X	NA	NA
Day 1: before dosing (All animals: Groups 1–8)	NA	NA	NA	X	X
Day 2 (All animals: Groups 1–8)	NA	NA	NA	NA	X
Day 3 (All animals: Groups 1–4)	NA	NA	NA	NA	X
Day 4 (All animals: Groups 1–8)	NA	NA	NA	NA	X
Day 4 (Main study animals: Groups 1–4)	X	NA	X	NA	NA
Day 15 (Recovery animals: Groups 3 and 4)	NA	NA	NA	X	X
Day 15: before dosing (All animals: Groups 5–8)	NA	NA	NA	X	X
Days 16 and 17 (All animals: Groups 5–8)	NA	NA	NA	NA	X
Day 18 (Main study animals: Groups 5–8)	X	NA	X	NA	X
Day 28 (Recovery Animal Nos. 3005, 3006, and 4005)	X	NA	X	NA	X
Day 29 (Recovery Animal Nos. 3007, 4006, and 4007)	X	NA	X	NA	X
Day 29 (Recovery animals: Groups 5, 7, and 8)	NA	NA	NA	NA	X
Day 57 (Recovery animals: Groups 5, 7, and 8)	NA	NA	NA	X	X
Day 74 (Recovery animals: Groups 5, 7, and 8)	X	NA	X	NA	X
Volume of Whole Blood	1.3 mL	1.3 mL	1.8 mL	1.0 mL	2.0 mL
Anticoagulant	EDTA	Sodium Citrate	SST	SST	SST

(Sponsor's Table)

Clinical Chemistry

No abnormal findings.

Urinalysis

Not evaluated.

Gross Pathology

There were no drug-related effects on organ weights or appearance.

Histopathology

Adequate Battery: Yes

Tissues Collected and Examined at Necropsy

Administration site[a]	Intestine, colon
Adrenal gland	Intestine, duodenum
Animal identification	Intestine, ileum (with Peyer's patch[d])
Aorta (thoracic)	Intestine, jejunum
Bone, femur	Intestine, rectum
Bone, joint (femoral-tibial)[b]	Kidney (paired)
Bone, sternum	Lacrimal gland (paired)
Bone marrow, sternum	Liver
Brain:	Lung
Basal ganglia	Lymph node, mandibular
Brain stem	Lymph node, mesenteric
Cerebellum	Nerve, optic (paired)[c]
Corpus callosum	Nerve, sciatic
Cerebrum	Pancreas
Cerebral and frontal cortex	Parathyroid gland[d]
Hippocampus	Pituitary gland
Lateral ventricle	Prostate gland
Medulla oblongata	Salivary gland, mandibular (paired), parotid, sublingual
Midbrain	
Occipital cortex	Seminal vesicle (paired)
Olfactory bulb	Skeletal muscle (biceps femoris, psoas)
Piriform lobe	Skin
Pons	**Spinal cord (cervical, thoracic, lumbar)**
Substantia nigra	Spleen
Temporal lobe	Stomach (cardiac, fundic, pyloric)
Thalamus	Testis (paired)[e]
Epididymis (paired)	Thymus
Esophagus	Thyroid gland (paired)
Eye (paired)[c]	Tongue
Gallbladder	Trachea
Heart	Urinary bladder
Intestine, cecum	Gross lesions/masses

[a] The final administration site was the interscapular region last used for SC dosing.
[b] The femoral-tibial joint was evaluated macroscopically, collected, and retained for possible histologi processing and examination.
[c] Fixed in Davidson's Solution.
[d] Examined only when present in the routine section.
[e] Fixed in Modified Davidson's Solution.
Bolded tissues constitute those required for a limited histopathology.

(Sponsor's Table)

Peer Review: Yes

Signed Pathology Report: Yes

Histological Findings:
Microglial aggregates, mixed cell infiltrates, and signs of microhemorrhage were observed in the brain and spinal cords of the HD animals on study Days 4 (Group 4; single dose) and 18 (Group 8; two doses). There was no neuronal necrosis. Immunostaining revealed that the glial cell aggregates were IBA-1^{+} and GFAP^{-}, indicating the presence of microglia, but not astrocytes. The mixed cell infiltrates included lymphocytes and neutrophils. IBA-1 staining at Day 18 also revealed locally extensive areas of increased staining in the parenchyma in Group 8 animals, suggesting microglial activation. Microglial aggregates, mixed cell infiltrates, and evidence of hemorrhage were less frequent after 4- and 8-week recovery periods in Groups 4 and 8, respectively, but failed to completely resolve.

Text Table 12
Incidence of DAC HYP-related Microscopic Findings in the Brain and Spinal Cord at Main Study Euthanasia(s)

	Day 4				Day 18			
	Vehicle	10 mg/kg	35 mg/kg	200 mg/kg	Vehicle	10 mg/kg	35 mg/kg	200 mg/kg
H&E-stained Slides:	n = 4	n = 4	n = 4	n = 4	n = 4	n = 4	n = 4	n = 4
Infiltrates, Mixed Cell (various locations in the brain, spinal cord, and meninges)	0	0	0	2	0	0	0	2
Glial Aggregates	0	0	0	3	0	0	0	3
Hemorrhage	0	0	0	2	0	0	0	1
Immunohistochemical Staining:	n = 2	n = 0	n = 0	n = 2	n = 0	n = 0	n = 0	n = 2
Glial Aggregates(s), IBA-1 Positive	0	-	-	2	-	-	-	2
Parenchyma, Increased IBA-1 Staining	0	-	-	0	-	-	-	2

- = No slides from animals in this group were stained.

(Sponsor's Table)

Text Table 13
Incidence of DAC HYP-related Microscopic Findings in the Brain and Spinal Cord at Recovery Euthanasia(s)

	Day 28/29[a]				Day 74[a]			
	Vehicle	10 mg/kg	35 mg/kg	200 mg/kg	Vehicle	10 mg/kg	35 mg/kg	200 mg/kg
	n = 3	n = 3	n = 3	n = 3	n = 3	n = 3	n = 3	n = 3
Infiltrates, Mixed Cell	-	-	0	1	0	-	0	0
Glial Aggregates	-	-	0	1	0	-	0	1
Hemosiderin	-	-	0	0	0	-	0	1

[a] Only H&E staining of slides was conducted for recovery animals.
- = No recovery animals in this group.

(Sponsor's Table)

Special Evaluation: Neurobehavioral Assessments

Neurobehavioral assessments were performed prior to the initiation of dosing, and on Days 4, 10, and 18, and during Week 11. Behavior was evaluated by observing each animal from a distance, then moving closer to observe the animal for awareness, curiosity, alertness, attentiveness, and response to the examiner's movement. Motor function was evaluated by observing symmetrical movement, strength, and coordination. Proprioception was evaluated by observing postural and gait reactions. Cranial nerve evaluation was performed by close visual inspection (see below) with the animal squeezed to the front of the cage. There were no abnormal findings.

Nerve		Functions Assessed
II	Optic	Vision
III	Oculomotor	Movements of the eye
IV	Trochlear	Eyelid
VI	Abducens	Response
V	Trigeminal	Mastication, jaw tone
VII	Facial	Facial expression
VIII	Vestibulocochlear/Auditory	Hearing and maintenance of head stability
X	Vagus	Breathing

(Sponsor's Table)

Toxicokinetics

Increases in DAC-HYP exposure were generally dose proportional, and exposure increased with multiple doses.

Dose (mg/kg)	Single Dose			Multiple Dose					
	Day 1			Day 1			Day 15		
	C_{max} (ng/mL)	T_{max} (days)	AUC_{D0-4} (ng×day/mL)	C_{max} (ng/mL)	T_{max} (days)	AUC_{D0-4} (ng×day/mL)	C_{max} (ng/mL)	T_{max} (days)	AUC_{D15-18} (ng×day/mL)
10	119750	3.5	302813	111325	4	1283588	186500	17.8	441575
35	413857	3.43	1072500	390143	3.71	116000	624286	17.1	1529446
200	1944286	3.71	299000	2790000	3.71	578000	3882857	17.3	28820714

Dosing Solution Analysis

Dosing solutions were between 98.9% and 105% of the target concentrations.

7 Reproductive and Developmental Toxicology

7.1 Fertility and Early Embryonic Development

Study title: A Male Reproductive Toxicology Study of Daclizumab Administered Bi-Weekly by Subcutaneous Injection to Cynomolgus Monkeys, with a Recovery Period.

Study no.:	TR07135_2
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	6/20/2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DAC-HYP, Lot #90670501, 99.0%

Methods

Doses:	0, 10, 50, 200 mg/kg
Frequency of dosing:	Every two weeks for 5 doses
Dose volume:	2 mL/kg
Route of administration:	SC injection
Formulation/Vehicle:	Preformulated in (b) (4) mM sodium succinate, (b) (4) mM sodium chloride, (b) (4) % Tween 80.
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	5/group (main); 3/group (12-week recovery)
Satellite groups:	None
Study design:	Male reproductive capacity was measured by surrogate markers, rather than mating. Markers included sperm quality and serum testosterone levels, which were measured twice prior to dosing, at the ends of months 1 and 2, and near the end of the recovery period.
Deviation from study protocol:	There were no significant deviations to the protocol. A pathology working group was convened to characterize the microglial aggregates found in the brain and spinal cord.

Summary of Findings

(Based on review of IND (b) (4) by T. Robison, Ph.D., 3/4/2008)

All animals survived until scheduled necropsy, with no abnormal clinical signs. There were no drug-related effects on sperm motility, concentration, or morphology, or testosterone concentrations. The NOAEL for fertility was 200 mg/kg (AUC = 784.1 mg×h/mL). However, the NOAEL based on the microglial aggregates is 10 mg/kg (AUC = 41.6mg×h/mL).

Microglial aggregates were randomly scattered and were not immunopositive for GFAP. Aggregates were often accompanied by mild focal or multifocal perivascular infiltrate of mononuclear inflammatory cells. Fluoro-Jade B staining did not reveal any evidence of neuronal degeneration. Microglial aggregates were not observed at the end of the recovery period.

Histopathological changes at end of the treatment (day 64) and recovery (day 142) periods

Organ/Tissue	End of the treatment period (Day 64)				End of the recovery period (day 142)	
	Control	10 mg/kg	50 mg/kg	200 mg/kg	Control	200 mg/kg
Brain						
-gliosis, multifocal, minimal	0/5	0/5	2/5	5/5	0/3	0/3
-infiltrate, mononuclear, perivascular, multifocal, minimal-mild	2/5	1/5	5/5	4/5	3/3	1/3
Spinal cord, thoracic						
-gliosis, white matter, multifocal	0/5	0/5	1/5	0/5	0/3	0/3
Injection Site						
-edema, perivascular, subcutaneous, multifocal, mild	0/5	0/5	0/5	1/5	0/3	0/3
-infiltrate, mononuclear cell, perivascular, subcutaneous, multifocal, minimal-mild	0/5	0/5	2/5	2/5	0/3	0/3
Colon						
-hemorrhage, lamina propria, multifocal	0/5	0/5	0/5	1/5	0/3	0/3
Stomach						
-hemorrhage, lamina propria, multifocal	0/5	0/5	1/5	1/5	0/3	0/3
LN Axillary						
-pigment, macrophage, sinus, multifocal	0/5	0/5	0/5	1/5	0/3	0/3
-plasmacytosis	1/5	0/5	0/5	0/5	1/3	3/3
-histiocytosis, sinus	1/5	1/5	0/5	1/5	1/3	3/3
-sinus erythrocytosis	1/5	0/5	1/5	2/5	1/3	2/3
LN, Mesenteric						
-hyperplasia, lymphoid	0/3	0/3	0/3	1/3	1/3	3/3
Mammary gland						
-vacuolation, cytoplasm, duct, epithelium, multifocal	1/5	0/5	0/4	2/3	0/3	0/3
Cecum						
-hyperplasia, lymphoid, gut-associated lymphoid tissue	0/5	0/5	0/5	0/5	0/3	1/3
Kidney						
-degeneration, mucinous, nerve fiber, pelvic	0/5	0/5	0/5	0/5	0/3	1/3

(Table from IND [redacted] nonclinical review)

The $t_{1/2}$ for DAC-HYP was 400.8 h in the HD animals. Exposure (C_{max} and AUCτ) was generally dose-proportional between the 1st and 4th doses, although increases in these parameters indicated accumulation to achieve steady-state levels. Anti-drug antibodies were detected in one HD animal prior to the start of dosing but were not detected subsequently.

Dose Group & Regimen	Statistics	C_{max} (mcg/mL)		t_{max} (hr)		AUC_{τ} (hr*mcg/mL)		Terminal $t_{1/2}$ (hr)
		1st dose	4th dose	1st dose	4th dose	1st dose	4th dose	
2 10 mg/kg: SC once every 2 weeks x 5	Mean	72.43	148.01	129.6	81.6	19785	41623	NA
	SD	9.83	29.37	52.6	64.8	3353	7636	NA
	n	5	5	5	5	5	5	NA
3 50 mg/kg: SC once every 2 weeks x 5	Mean	346.58	871.64	57.6	67.2	95148	211945	NA
	SD	62.32	108.13	13.1	10.7	11896	19789	NA
	n	5	5	5	5	5	5	NA
4 200 mg/kg: SC once every 2 weeks x 5	Mean	1772.39	3193.13	96.0	60.0	450312	784071	400.8
	SD	642.75	710.16	44.4	12.8	119169	164147	97.9
	n	8	8	8	8	8	8	3

(Sponsor's Table)

Study title: A Female Reproductive Toxicology Study of Daclizumab Administered Bi-Weekly by Subcutaneous Injection to Cynomolgus Monkeys, with a Recovery Period.

Study no.: TR06121-1
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 5/18/2005
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: DAC-HYP, Lot #90670404, 99.3%

Methods

Doses:	0, 10, 50, 200 mg/kg
Frequency of dosing:	Every two weeks for 5 dosing periods (Study Days 1, 15, 29, 43, and 57).
Dose volume:	2 mL/kg
Route of administration:	SC injection
Formulation/Vehicle:	Preformulated in (b) (4) mM sodium succinate, (b) (4) mM sodium chloride, (b) (4) % Tween 80.
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	5/group (main); 3/group (12-week recovery)
Satellite groups:	None
Study design:	The objective of this study was to evaluate any effects of DAC-HYP on the menstrual cycle, and reproductive hormones and organs in female monkeys. Dosing extended over 2 menstrual periods, and was synchronized to relative cycling.
Deviation from study protocol:	There were no significant deviations.

Observations and Results

Mortality

All animals survived until scheduled necropsy.

Clinical Signs

Twice daily cage side observations did not reveal any drug-related clinical signs.

Body Weight

All animals were weighed weekly; there were no drug-related effects on weight gain.

Food Consumption

Food consumption was monitored daily; there were no drug-related effects.

Toxicokinetics

TK parameters were calculated after the 1st and 5th dose. Exposure (C_{max} and AUC) increased in a dose-related manner, although accumulation by the 5th dose is indicated by elevations in exposure. The elimination half-life ranged from 268 to 313 h. Anti-daclizumab antibodies were found in LD (2/5) and MD (1/5) animals.

	1st dose interval			5th dose interval			
Group/Dose Level	t_{max} (hr)	C_{max} (mcg/mL)	AUC_τ (hr·mcg/mL)	t_{max} (hr)	C_{max} (mcg/mL)	AUC_τ (hr·mcg/mL)	$t_{1/2}$ (hr)
2: 10 mg/kg	130	98.2	26,921	38	141.8	39,626	267.8
3: 50 mg/kg	91	397.5	107,760	48	738.8	193,403	352.4
4: 200 mg/kg	84	1,608.0	394,373	43	2,609.7	647,282	312.7

(Sponsor's Table)

Dosing Solution Analysis

An aliquot from each dosing solution was returned to the sponsor to confirm formulation accuracy. With the exception of two instances, all dosing solutions were within 10% of the target concentration. The doses given to female Nos. 41 (Group 2, Day 1) and 21 (Group 4, Day 57) were ↑15% and ↑12% of their respective target concentrations.

Menstrual Cycle Length and Endocrinology

With the exception of one HD female (No. 8), there were no abnormal menstrual or estradiol/progesterone patterns. Although Female No. 8 demonstrated regular menstrual cycles prior to assignment, complete cessation of menstrual and ovarian function occurred after dosing. The abnormal menstrual cycle in Female No. 8 correlated with abnormal histology findings (see below) and was not likely drug-related.

Necropsy

There were no abnormal serum chemistry or hematology results. No gross findings were apparent in the main or recovery animals, with the exception of Female No. 8, which had bilaterally enlarged ovaries and oviducts, with multiple cysts in each ovary. Microscopic evaluation revealed the cysts to be filled with a proteinaceous fluid resembling thyroid colloid. A pathology working group concluded that the observations in Female No. 8 resembled serous cystadenoma in humans, which is a benign ovarian tumor and was likely present before dosing began. No other abnormal findings in the reproductive organs were reported. Histologic evaluation was limited to the reproductive organs; no CNS evaluation (i.e., microglial aggregates) was performed.

7.2 Embryonic Fetal Development

Study title: Preliminary Embryo-Fetal Development Study of Daclizumab Administered by Subcutaneous Injection to Pregnant Cynomolgus Monkeys.

Study no.:	TR07122
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	11/1/2004
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	DAC-HYP, Lot #90670403, 99.1%

Summary of Findings

(Based on review of IND (b) (4) by T. Robison, Ph.D., 3/4/2008)

The objective of this preliminary embryo-fetal study was to evaluate the effects of DAC-HYP on embryonic and fetal development. However, no control group was included in

this study, potentially complicating the evaluation of any abnormalities. 5 female cynomolgus monkeys received DAC-HYP at a dose of 200 mg/kg/week from GD 20 or 22 to GD 50. All 5 females maintained pregnancy until the day of C-section. No abnormal measurements were observed by routine ultrasounds throughout the study. No external or visceral abnormalities were observed in the 5 male fetuses.

Study title: Embryo-Fetal Development Study of Daclizumab Administered by Subcutaneous Injection to Pregnant Cynomolgus Monkeys.

Study no.:	TR07123
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	4/6/2005
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Daclizumab, Lot # 90670403, 99.1%

Methods

Doses:	0, 10, 50, 200 mg/kg
Frequency of dosing:	Once weekly, for 5 doses
Dose volume:	2 mL/kg
Route of administration:	SC injection
Formulation/Vehicle:	Preformulated in (b) (4) mM sodium succinate, (b) (4) mM sodium chloride, (b) (4) % Tween 80.
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	12-15 females/group
Satellite groups:	None
Study design:	Pregnant cynomolgus monkeys received the first daclizumab or control dose within 2 days of confirmed pregnancy (GD 20-22), and weekly thereafter. Fetuses were harvested by Cesarean section at approximately GD 100, and placental, external, visceral, cardiac, and skeletal evaluations were performed.
Deviation from study protocol:	There were no significant protocol deviations.

Observations and Results

Mortality

An increased incidence of fetal loss was observed in the HD group. With the exception of one death in the MD group, all fetal deaths occurred prior to GD 50. Following an emergency C-section, the cause of death for one HD fetus was thought to be related to an abnormal placenta. The cause of death was undetermined for the remaining fetal losses. In the MD group, the fetal death that occurred on GD78 was possibly due to the

small size of the pregnant female. The increased incidence of fetal loss in the HD group was stated to be approximately twice the historical control rate and was likely drug-related.

Group	Dose Level	Total No. Pregnant Females Enrolled	Gestation Day of Fetal Loss (Adult Female No./ Fetus No.)	Percent Loss by Dose Level
1	Control (0 mg/kg)	13	GD32 (144/NA)	7.7%
2	Daclizumab (10 mg/kg)	13	GD32 (110/NA)	7.7%
3	Daclizumab (50 mg/kg)	12	GD78 (47/9142)	8.3%
4	Daclizumab (200 mg/kg)	15	GD33 (194/NA) GD46 (139/NA) GD47 (181/9141)	20%

(Sponsor's Table)

Clinical Signs

Cage side observations were performed twice daily prior to pregnancy and up to the scheduled Cesarean section. Sporadic findings of low food consumption, dry skin, emesis, alopecia, and watery stool occurred throughout all groups, including controls.

Body Weight

Body weight was measured weekly prior to pregnancy and up to the scheduled Cesarean section. There were no drug-related effects on weight gain.

Food Consumption

Food consumption was measured daily prior to dosing and up to the scheduled Cesarean section. There were sporadic occurrences of low food consumption across all groups.

Toxicokinetics

TK parameters were calculated after doses 1 and 5. Increases in exposure (C_{max} and AUC) were generally dose proportional on both days. Maximal plasma concentration (T_{max}) occurred between 2.9 and 4.0 days after the first dose, and 1.9 and 2.2 days after the 5th dose. The terminal half-life ($t_{1/2}$) ranged from 7.3 to 8.8 days. Four LD females were positive for anti-Daclizumab antibodies, but only one animal showed pronounced reductions in serum drug levels. Based on fetal loss, the NOAEL for this study was 50 mg/kg Q1W (AUC_{τ} = 128 mg×h/mL).

Summary of Main Toxicokinetic Parameters (Group Mean)

Dose Group & Regimen	Statistics	C_{max} (mcg/mL)		t_{max} (day)		AUC_τ (hr*mg/mL)		Terminal $t_{1/2}$ (day)
		1st Dose	5th Dose	1st Dose	5th Dose	1st Dose	5th Dose	
2 10 mg/kg; SC weekly x 5	**Mean**	**85**	**183**	**4.0**	**2.2**	**12**	**24**	**7.3**
	SD	25	81	1.8	0.6	3	11	2.6
	%CV	29	44	44	27	27	44	36
	n	13	12	13	12	13	12	12
3 50 mg/kg; SC weekly x 5	**Mean**	**384**	**930**	**3.4**	**1.9**	**55**	**128**	**8.8**
	SD	82	267	2.1	1.3	11	33	1.9
	%CV	21	29	60	70	20	26	22
	n	12	12	12	12	12	12	12
4 200 mg/kg; SC weekly x 5	**Mean**	**1857**	**3794**	**2.9**	**2.0**	**247**	**533**	**8.8**
	SD	194	509	1.0	0.9	25	65	1.0
	%CV	10	13	35	44	10	12	11
	n	15	12	15	12	15	12	12

AUC_τ: area under concentration-time curve within a dosing interval
C_{max}: observed maximum serum concentration within a dosing interval
SC: subcutaneous injection
$t_{1/2}$: half life
t_{max}: time at which the maximum concentration was observed

(Sponsor's Table)

Dosing Solution Analysis

Daclizumab (b) (4) was supplied preformulated. Fresh vials were used on each day of dosing. Samples from each dosing solution were returned to the sponsor for evaluation. All dosing solutions were within 10% of the target concentration.

Clinical Chemistry

Analysis of serum chemistry and hematology were performed throughout gestation (GD 20-22, GD 53-55, and GD 98-102); there were no remarkable findings.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

Ultrasound evaluations throughout gestation revealed an abnormally small fetus in 1 MD animal on GD 74, which was accompanied by decreases in amniotic fluid volume by GD 88. Following C-section, the small size of the fetus was thought to be due to reduced amniotic fluid volume (2 mL) and a twisted umbilical cord. The reduced size of this fetus resulted in decreases in mean fetus measurements in the MD group.

Dose (mg/kg)	0	5	50	200
Fetal Measurements				
Sex:				
male	10	5	5	
female	2	7	6	2
Crown-to-Rump	--	--	↓7%	↓10%
Crown-to-Heel	--	--	--	↓5%
Foot Length	--	↓3%	↓8%	↓4%
Body Weight	--	--	↓11%	↓3%
Body Weight				

Offspring (Malformations, Variations, etc.)

Fetal skeletal examination did not reveal any drug-related bone abnormalities. The following fetal tissues were harvested at C-section but not evaluated.

Tissues Collected	
Cardiovascular	Urogenital
Heart with Aorta	Kidneys
Digestive	Urinary Bladder[a]
Esophagus	Testes[b]
Stomach[a]	Epididymis[b]
Small Intestine[a]	Prostate[a]
Duodenum[a]	Seminal Vesicles[a]
Jejunum[a]	Ovaries[a]
Ileum[a]	Uterus[a]
Large Intestine[a]	Cervix[a]
Cecum[a]	Vagina[a]
Colon[a]	Oviduct[a]
Rectum[a]	Endocrine
Pancreas[a]	Adrenals
Liver/Gallbladder	Pituitary
Respiratory	Thyroid/Parathyroids[c,d]
Diaphragm	Nervous/Special Sense
Lung	Eyes
Trachea[c]	Brain
Lymphoid/Hematopoietic	Other
Thymus	Gross Lesions
Spleen	Fetal Skeleton
	Placenta
	Umbilical Cord

[a] These tissues could have been collected together en masse.
[b] These tissues could have been collected together.
[c] These tissues could have been collected together.
[d] The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.

(Sponsor's Table)

7.3 Prenatal and Postnatal Development

Study title: Pre-Postnatal Toxicity of Daclizumab HYP (b) (4) Administered by Subcutaneous Injection to Pregnant Cynomolgus Monkeys with a 6-Month Postnatal Evaluation

Study no.:	TC11-033
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/9/2011
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Daclizumab, lot #VVKD72, 99.1%

Methods

Doses:	0, 50 mg/kg
Frequency of dosing:	1/week from GD 50 to approximately GD 160
Dose volume:	0.33 mL/kg
Route of administration:	SC injection
Formulation/Vehicle:	Preformulated by the sponsor in a ready-to-use state. Dosing solutions were within 91.4-101% of the target concentration.
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	20 pregnant females/group
Satellite groups:	None
Study design:	The toxicity of daclizumab was evaluated in pregnant monkeys. Offspring were delivered naturally on GD 160 ±10, and the adults and offspring were monitored for an additional 6 months for adverse drug effects.
Deviation from study protocol:	There were no significant deviations from the protocol.

Results.
The objective of this study was to evaluate the toxicity of DAC-HYP in pregnant cynomolgus monkeys and their offspring. There were no effects of daclizumab on gestation length. Pregnancy and infant losses are summarized in the table below.

Group	No. of Pregnant Females	No of Live Infants (M/F)	Pregnancy and Infant Losses (mother/fetus or infant)	Day of Loss	Cause of Death
Control	20	17 (9/8)	1520/1206	GD114	Unknown
			1507/1071	GD121	Unknown
			1523/1236	GD147	Unknown
			1517/1171	PND2	Unknown
			1511/1111	PND 6	Euthanized due to maternal mistreatment
			1504/1041	PND 18	Euthanized due to poor maternal care/bone fracture
DAC-HYP (50 mg/kg)	20	19 (10/9)	2510/2100	GD117	Unknown
			2512/2126	PND 1	Euthanized due to poor maternal care
			2517/2171	PND 2	Poor nursing interaction
			2521/2216	PND 2	Born with facial lacerations that interfered with nursing; euthanized
			2514/2146	PND 3	Euthanized due to maternal neglect
			2518/2181	PND 10	Euthanized due to maternal mistreatment
			2520/2201	PND 15	Delivered preterm; euthanized due to maternal mistreatment
			2503/2036	PND 29	Euthanized due to trauma (broken femur)

There were no drug-related clinical signs or changes in weight gain in the adults during gestation or following parturition. Routine ultrasounds throughout gestation did not reveal any effect on heart rate in the fetuses. There were no drug-related clinical signs or effects on weight gain or morphometric measurements in the offspring at birth or during the 6-month observation period. Neurobehavioral assessments were performed on BD 7 and 14, and did not reveal any drug effects on righting, palmer grasp, clasp-grasp, visual following, prone progression, lipsmack orient, oral reflexes, eye reflexes, moro reflexes, negative geotaxis, buildup (increasing arousal levels in response to manipulation).

Hematology analysis performed on GD 50 and 134 in the adults and on BD 28, 91, and 180 in the infants did not reveal any drug-related findings.

Toxicokinetics

TK analysis was performed on blood samples from the adult animals on dosing Days 1 and 13. No analysis of DAC-neutralizing antibodies was performed.

Dosing Day	T_{max} (h)	C_{max} (μg/mL)	$AUC_{(0-7)}$ (μg×h/mL
1	82.2	485	65000
13	54.7	1450	209000

DAC-HYP was detected in milk from 9/14, 5/14, and 0/14 adults on PND 14, 28, and 91, respectively, but never exceeded 0.122% of the corresponding serum concentration.

Table 5. ABT-803 Milk/Serum Concentration Ratio During Lactation Period of Cynomolgus Monkey Adult Females

Time Hours	Animal 2501	Animal 2502	Animal 2503	Animal 2504	Animal 2505	Animal 2506	Animal 2507	Animal 2508	Animal 2509	Animal 2511	Animal 2513	Animal 2516	Animal 2519	Animal 2520
BD014 / Day 1 336h	NA	NA	0.00086	0.00122	NA	0.00047	0.00092	0.00080	NA	0.00028	0.00117	0.00079	NA	0.00104
BD028 / Day 1 672h	NA	0.00109	0.00114	NA	NA	0.00050	NA	0.00090	NA	NA	NA	NA	0.00084	NA
BD091 / Day 1 2184h	NA	NA	NA N	A	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

*NA = Ratio not available (details in Reference 2; e.g., for some samples quantity of milk not sufficient for assay, for others, result below quantitative limit or milk sample not analyzed due to presence of blood).

(Sponsor's Table)

Transplacental passage was evaluated by comparing DAC-HYP concentrations between an infant and its mother on PPD14, 28, and 91, during which the ratios increased from 1.0 to 2.3, indicating the infants might be eliminating daclizumab more slowly than the adults.

Table 4. Exposure Ratio of (b) (4) in Serum: Infant and Corresponding Mother After Birth

	Animal ID-Numbers																	
Time Standard	2016/ 2501	2026/ 2502	2036/ 2503	2041/ 2504	2051/ 2505	2066/ 2506	2071/ 2507	2081/ 2508	2096/ 2509	2116/ 2511	2126/ 2512	2131/ 2513	2146/ 2514	2161/ 2516	2191/ 2519	2201/ 2520	2216/ 2521	Mean	SD
PPD1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.0	NA	0.0	NA	NA	NA	NA		
PPD2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.7		
PPD3	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.8	NA	NA	NA	NA		
PPD14	1.5	0.6	0.4	NA	1.0	0.9	0.9	0.9	0.7	0.6	NA	0.7	NA	1.7	1.5	NA	NA	1.0	0.4
PPD15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	1.1	NA		
PPD18	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
PPD28	1.3	NA	0.7	0.8	1.2	0.8	0.9	1.1	1.0	0.5	NA	0.8	NA	2.8	1.2	NA	NA	1.1	0.6
PPD29	NA	NA	0.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		
PPD91	4.8	0.9	NA	1.8	2.5	1.7	1.0	4.4	0.4	0.2	NA	1.2	NA	8.3	1.0	NA	NA	2.3	2.4
PPD180	NA	NA	NA	NA	NA	NA	NA	NA	0.1	NA	NA	NA	NA	NA	NA	NA	NA		

*NA = no serum samples available

(Sponsor's Table)

Serum Immunoglobulin, Peripheral Blood Immunophenotyping, and T-cell Dependent Antibody Response

Serum IgM and IgG levels were evaluated in adults on GD 50 and 134 and in infants on BD14, 28, 91, and 180; there were no drug-related changes. There were no DAC-HYP related changes in CD20+ B-lymphocytes, CD3+ T-lymphocytes, CD3+/CD4+ T-helper lymphocytes, CD3+/CD8+ T-cytotoxic lymphocytes, CD3-/CD14+ monocytes, or CD3-/CD16+ natural killer cells in infants on BD 28, 91, and 180. T-cell dependent antibody response was evaluated on BD147, 154, 161, 168, and 175 in the infants, following an intramuscular injection of keyhole limpet hemocyanin (KLH) on BD147; there were no daclizumab-related changes in anti-KLH IgM or IgG.

Necropsy
A complete necropsy was performed on all infants, and the following tissues harvested and preserved in 10% neutral-buffered formalin. There were no remarkable findings.

Brain	Lymph node, mandibular
Gross lesions/masses (unscheduled only)	Lymph node, mesenteric
Heart	Lymph node, popliteal
Kidney	Lymph node, trachea-bronchial
Liver	Spinal cord
Lung	Spleen
Lymph node, axillary	Thymus

8 Special Toxicology Studies

Study title: Characterization of Interleukin 2 (IL-2) receptor protein expression and IL-2 proliferation responses by primary human microglial cells.

Study no.:	R&D-13-953
Study report location:	EDR
Conducting laboratory and location:	AbbVie Biotherapeutics Corp. 1500 Seaport Blvd Redwood City, CA 94063
Date of study initiation:	6/4/2012
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	DAC-HYP was not used in this study.

Objective/Methods:

The objective of this *in vitro* study was to determine whether the microglial aggregates observed in the nonclinical studies are due to direct effects of DAC-HYP on microglial cells, or whether the effect is an indirect result of elevations in IL-2 bioavailability within the CNS. Support for the second hypothesis is based on reported elevations in serum IL-2 levels in MS patients receiving DAC-HYP, probably due to suppression of the high-affinity CD25 receptors. However, serum IL-2 was never measured in the monkeys.

Using cultured human microglial cells, the sponsor tested 1) expression of CD25, 2) expression of the CD122 and CD132 subunits of the IL-2 receptor, 3) proliferation in response to IL-2 stimulation, and 4) effects of CD25-blocking antibody fragments on IL-2 mediated proliferation. At no point were the microglial cells actually treated with DAC-HYP to test a direct interaction.

Results:
The identity of the cultured microglial cells was confirmed by FACS analysis, using antibodies against the microglial markers CD45 and CD11b. The absence of CCR2 immunoreactivity was used to confirm that the CD45 and CD11b-immunoreactive cells were microglia, and not CNS resident monocytes. FACS analysis was also used to demonstrate that the microglial cells did not express the CD25 (high-affinity) subunit of the IL-2 receptor but did express CD122 and CD132 (intermediate affinity). IL-2 induced proliferation of microglia was measured by ^{3}H thymidine incorporation in the presence of increasing concentrations of IL-2, revealing a dose-dependent effect that was not suppressed by CD25 blockade using a variant of daclizumab that lacks the Fc region. Although the microglial cells did not appear to express CD25, it was unclear why a direct interaction with DAC-HYP, such as through the FcR, was not evaluated, especially since the pharmacology studies indicated affinity for FcγIIIR (Study RTR41). These data support the sponsor's hypothesis that the microglial aggregates are due to elevations in IL-2 concentration, but alternative mechanisms, such as a direct interaction between DAC-HYP and microglia, cannot be ruled out based on this study.

Study title: Characterization of Interleukin 2 (IL-2) receptor protein expression and IL-2 proliferation responses by primary cynomolgus monkey microglial cells.

Study no.:	R&D-13-970
Study report location:	EDR
Conducting laboratory and location:	AbbVie Biotherapeutics Corp. 1500 Seaport Blvd Redwood City, CA 94063
Date of study initiation:	10/22/2012
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	DAC-HYP was not used in this study.

Objective/Methods:

The objective of this study was to determine whether primary microglial cells isolated from cynomolgus monkeys express CD25, and are therefore susceptible to direct interaction with DAC-HYP. However, a direct interaction between the cultured microglial cells and DAC-HYP was never evaluated. In isolated primary microglia isolated from cynomolgus monkeys, the sponsor tested 1) CD25 expression, 2) CD123 and CD132 expression, and 3) microglial proliferation in response to IL-2.

Results:
Following isolation from monkey brains using CD11b-linked beads, microglial cultures were evaluated by FACS analysis for immunoreactivity with CD45 and CD11b, indicating that the culture was primarily microglial cells. FACS analysis also demonstrated the absence of GLAST (astrocyte marker) immunoreactivity in the CD45 and CD11-positive cells, further indicating that the culture was primarily microglia and not astrocytes. Using antibodies against CD25, the sponsor indicated an absence of the high-affinity subunit of the IL-2 receptor in the cultured microglia. However, at no point was labeling with DAC-HYP performed. It was unclear if the CD122 and CD132 were expressed by the monkey microglia, since binding by antibodies generated against human CD12 and CD132 was minimal. Culturing the microglial cells in the presence of increasing IL-2 concentrations resulted in a dose-dependent increase in ^{3}H-thymidine incorporation. Incubation of the microglial cells with human IgG or a DAC-HYP fragment which lacks the Fc region did not suppress IL-2 mediated proliferation. The sponsor justified the use of the Fc-deficient fragment based on the abundance of Fc receptors on the microglial cells. This study failed to address a direct interaction between DAC-HYP and microglial cells.

Study title: Local Tolerance Study in Rabbits (Intravenous Injection) with Daclizumab.

Study no.:	TR06098
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3/8/2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DAC-HYP, Lot #90670404, 99.3%

Results:

The objective of this study was to evaluate the local tolerance of DAC-HYP. Six female NZW rabbits received a single 25 mg dose of daclizmab by bolus injection in the right ear; control article was administered in the left ear. Mortality, clinical observations, and local irritation were monitored, and 2 animals were necropsied at 30 min, 3 h, and 24 h post dose.

All rabbits survived until scheduled necropsy. Slight erythema was noted in one animal at the 30 min interval. No inflammatory infiltrate was observed by microscopic evaluation of any of the injections sites. Moderate to moderately-severe hemorrhage was observed at the DAC-HYP injection site in 5/6 animals. Minimal or slight hemorrhage was observed at the control injection site in 3/6 animals. The hemorrhage was thought to be due to disruption of the vessel wall integrity during the injection process.

DAC-HYP was not considered to cause irritation when administered IV.

Study title: Hemolysis assay in human whole blood with daclizumab.

Study no.:	TR06080
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	3/10/2006
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DAC-HYP, Lot #90670404, 99.3%

Results:

DAC-HYP tested negative for hemolysis in human whole blood at concentrations of 25, 50, and 98 mg/mL.

Study title: Method Qualification Study to Establish the Conditions for Cross-Reactivity of Zenepax and Daclizumab HYP with normal Human and Cynomolgus Monkey Tissues.

Study no.:	TR08279
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	5/2/2008
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	DAC-HYP, Lot #90670405, 97.7%

Results:

The objective of this study was to develop a method to evaluate DAC-HYP binding in cryosections from human and monkey tissue. Sections of tonsil tissue were used for a positive control, while vascular smooth muscle cells, which do not express CD25, were visualized as a negative control. Staining concentrations of 5 and 20 μg/mL, precomplexed with a biotin-labeled secondary antibody, were determined to be adequate for evaluating daclizumab binding.

Study title: Cross-Reactivity Study of Daclizumab HYP with Normal Human and Cynomolgus Monkey Tissues.

Study no.:	TR08278
Study report location:	EDR
Conducting laboratory and location:	(b) (4) [redacted]
Date of study initiation:	5/2/2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	DAC-HYP, Lot #90670405, 97.7%

Results:

The cross-reactivity of daclizumab or a negative control antibody (HuIgG1) was evaluated in cryosections from the following tissues harvested from cynomolgus monkeys and humans.

Adrenal	Lung	Spinal Cord
Blood Cells[A]	Lymph Node	Spleen
Blood vessels (endothelium)	Ovary	Striated (skeletal) Muscle
Bone Marrow	Fallopian Tube (oviduct)	Testis
Brain – cerebrum (cortex)	Pancreas	Thymus
Brain – cerebellum	Parathyroid	Thyroid
Breast (mammary gland)	Peripheral Nerve	Tonsil
Eye	Pituitary	Ureter
Gastrointestinal Tract[B]	Placenta	Urinary Bladder
Heart	Prostate	Uterus- body (endometrium)
Kidney (glomerulus, tubule)	Salivary Gland	Uterus – cervix
Liver	Skin	

(Sponsor's Table)

In the monkey panel, DAC-HYP bound elements of all tissues except adrenal gland, bone marrow, cerebrum and cerebellum, kidney, ovary, parathyroid, and urinary bladder. In the human panel, DAC-HYP bound elements of all tissues except blood smears, bone marrow, and peripheral nerve. In CNS tissue from both species, DAC-HYP was bound to cytoplasmic granules in glial cells, which were thought to include both astrocytes and microglial cells.

9 Integrated Summary and Safety Evaluation

Introduction
Daclizumab-High Yield Process (DAC-HYP; ZINBRYTA™) is a humanized monoclonal antibody developed by Biogen Idec Inc. for the treatment of relapsing forms of multiple sclerosis (MS). The proposed clinical dose for DAC-HYP is 150 mg administered once every four weeks by subcutaneous injection with a prefilled syringe.

Pharmacology
DAC-HYP is thought to suppress T-cell mediated autoimmunity in MS by selectively binding and inhibiting the CD25 subunit of the high-affinity IL-2 receptors. DAC-HYP does not suppress the intermediate (i.e. non-CD25) IL-2 receptors. Pharmacology studies demonstrated that DAC-HYP blocks IL-2 induced proliferation and cytokine secretion in primary lymphocyte cultures from humans and cynomolgus monkeys. However, DAC-HYP was not pharmacologically active in rodent lymphocyte cultures. Plasmon surface resonance revealed similar affinity for human (K_D=0.49 nM) and monkey (K_D=0.42 nM) CD25. DAC-HYP induced antibody-dependent cell-mediated cytotoxicity (ADCC) in a cell culture system, likely through interaction with $F_C\gamma$III receptors on NK cells. DAC-HYP did not induce complement-mediated cell death. Given the absence of pharmacological activity in rodents, the *in vivo* toxicology studies were performed in monkeys.

PK/ADME
A standard battery of PK/ADME studies were not conducted for DAC-HYP. Analytical methods validated for the nonclinical studies included ELISA protocols for measuring DAC-HYP in serum and milk samples, a competition ECL assay for detecting anti-daclizumab antibodies, and a flow cytometry method for detecting anti-daclizumab antibodies. TK data were collected in the toxicology studies. Following SC administration in monkeys, bioavailability ranged from 50 to 73%, with a T_{max} of 2-3 days and $t_{1/2}$ of approximately 10 days. Exposure (C_{max} and AUC) was consistent between males and females, and increased in a dose-proportional pattern.

Additional Studies
There was no irritation when DAC-HYP was administered to rabbit ears by IV injection. There was no hemolysis of human whole blood exposed to DAC-HYP. In cross-reactivity studies, DAC-HYP did not bind adrenal, bone marrow, cerebrum, cerebellum, kidney, ovary, parathyroid, and urinary bladder tissues in monkeys, or blood smears, bone marrow, and peripheral nerve tissues in humans. In both species, DAC-HYP was bound to cytoplasmic granules in glial cells, which were thought to include both astrocytes and microglial cells.

Toxicology
No adverse clinical signs were observed in monkeys administered a single IV bolus of 15 or 30 mg/kg DAC-HYP. Repeat-dose toxicology was initially evaluated through biweekly SC injection of up to 200 mg/kg DAC-HYP over 6 or 13 weeks in male and

female monkeys. Pivotal toxicology was first evaluated with a 39-week study in male and female monkeys administered 0, 10, 50, or 200 mg/kg DAC-HYP by biweekly SC injection. A second study of similar design but of 9-months duration was later conducted to evaluate doses of 0, 10, 35, or 200 mg/kg. Target organs for drug-related toxicity in the repeat-dose studies included the skin and CNS. Following the identification of CNS toxicity, a 2-week study consisting of one or two SC doses was performed to better evaluate acute CNS toxicity.

Dermal Toxicity:
Reversible, drug-related dermal effects were observed in males and females in the 39-week and 9-month studies. Skin findings consisted of increased frequency and severity of dry, red, raised patchy areas of skin, with microscopic correlates consisting of minimal to mild acanthosis/hyperkeratosis and inflammation. No mechanism for the dermal toxicity was described by the sponsor, although it was suggested that elevations in serum IL-2 levels may play a role in this finding. Although elevations in serum IL-2 were reported following DAC-HYP administration in humans, no such measurements were performed in monkeys. There was no NOAEL for the dermal toxicity.

Central Nervous System Toxicity
Microglial aggregates in male and female monkey brain and spinal cord were reported at doses greater than 10 mg/kg in the 13-week, 39-week, and 9-month studies. Similar findings were also reported in the male fertility study at doses greater than 10 mg/kg. The microglial aggregates consisted of small (≤30 cells) accumulations of cells distributed randomly throughout the brain (gray and white matter) and the spinal cord. Aggregates were often accompanied by inflammatory infiltrate and occasionally by signs of microhemorrhage (hemosiderin deposits). The NOAEL for microglial aggregates was 10 mg/kg in the 39-week and 9-month studies.

An acute study consisting of only 1 or 2 doses (0, 10, 35, 200 mg/kg SC) was performed to better characterize the CNS toxicity of DAC-HYP. Microglial aggregates appeared after a single 200 mg/kg dose. Immunostaining confirmed that the aggregates consisted primarily of microglial cells (i.e., $IBA1^+$) and not astrocytes. No signs of demyelination or axonal/neuronal degeneration accompanied the aggregates and general resolution was observed after a 4- or 12-week recovery period. A functional observation battery was performed to evaluate cranial nerve function, which did not reveal any behavioral abnormalities. However, it is unclear whether such a qualitative evaluation would be sensitive enough to detect a subtle neurological effect.

A mechanism responsible for the formation of the microglial aggregates has not been identified, but the sponsor has proposed that elevations in serum IL-2 with subsequent interactions with intermediate-affinity receptors on the microglia might lead to this finding. To support this argument, studies were conducted in isolated human or monkey microglial cells, revealing the presence of CD122 and CD132 (intermediate affinity), but not CD25 (high affinity) IL-2 receptors. Subsequent experiments with the microglial cultures revealed a proliferative response to IL-2, which was not suppressed by co-administration of a DAC-HYP fragment which lacks the F_C region. These data support

the sponsor's suggestion that microglial activation might occur in response to elevated IL-2 levels. However, IL-2 levels were never measured in the monkeys. Additionally, based on these experiments, a direct interaction between DAC-HYP and the microglial cells cannot be ruled out.

Reproductive and Developmental Toxicology
Biweekly SC administration of up to 200 mg/kg DAC-HYP for 5 doses did not affect sperm morphology in males or hormonal or menstrual cycles in females

Embryofetal development was evaluated in pregnant cynomolgus monkeys following weekly SC administration of up to 200 mg/kg DAC-HYP for 5 doses, resulting in a 20% (3/15) fetal loss in the HD group. This observation was twice the historical control rate and was possibly drug-related. Following C-section, there were no fetal malformations, but histopathology was not evaluated so it is unknown if microglial aggregates were present in the fetal brains.

Weekly SC administration of 50 mg/kg DAC-HYP from GD 50 to parturition (GD 160) did not have any effect on pre- or postnatal (PND 180) development. DAC-HYP was present in the milk of 11/14 dams at levels ≤0.122% of their respective serum concentrations. DAC-HYP was detected in infant plasma up to postpartum day 90, and infant:dam DAC-HYP plasma ratios ranged from 1 to 2. There were no adverse clinical signs in the infants, and no histological abnormalities, including microglial aggregates, were found in the brains or spinal cords following necropsy.

Juvenile Toxicology
The sponsor has proposed (b) (4)
(b) (4)
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However, after submitting this NDA, the sponsor requested a waiver for the juvenile study (b) (4)
(b) (4)

Conclusions
The dermal toxicity observed in nonclinical testing has been observed in clinical trials. However, it is unknown if microglial aggregates are similarly replicated in humans, since this finding cannot be monitored in a clinical setting. Concern over the microglial aggregates is heightened due to the unknown effect of this finding on any existing neuroinflammation in the intended patient population. Based on steady state exposures

in monkeys at the NOAEL and humans receiving the proposed clinical dose, the safety margin for the microglial aggregates is approximately 9-fold. Given an inadequate safety margin for the microglial aggregates and the availability of alternate therapy for relapsing forms of MS, DAC-HYP is not recommended for approval.

10 Appendix/Attachments

References

Rose, C, CM Luetjens, S Grote-Wessels, and GF Weinbauer, 2015, Feasibility of repeated testing for learning ability in juvenile primates for pediatric safety assessment, Regul Toxicol Pharmacol, 73:571-577.

Weinbauer, GF, A Fuchs, M Niehaus, and CM Luetjens, 2011, The Enhanced Pre- and Postnatal Study for Nonhuman Primates: Update and Perspectives, Birth Defects Res C Embryo Today, 93:324-333.

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/s/

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02/24/2016

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