3A Secondary Pharmacodynamics

In Vitro Immune Function Tests **Test Article:** natalizumab

Study No. PC032 **Location in CTD:** M4.2.1.1

	Anti-CD3 Stimulated Proliferation of PBMC (OD at ——)			
	0 ng/mL Anti-CD3	10 ng/mL Anti-CD3	100 ng/mL Anti-CD3	1000 ng/mL Anti-CD3
0 µg/mL	0.814 ± 0.084	1.174 ± 0.127	1.022 ± 0.132	0.999 ± 0.122
100 µg/mL[a]	0.945 ± 0.098	1.249 ± 0.121	1.140 ± 0.126	1.156 ± 0.106
	PHA Stimulated Proliferation of PBMC (OD at ——)			
	0 µg/mL PHA	0.5 µg/mL PHA	1 µg/mL PHA	5 µg/mL PHA
0 µg/mL	0.795 ± 0.094	0.849 ± 0.026	1.033 ± 0.043	1.352 ± 0.098
100 µg/mL	0.988 ± 0.104	0.984 ± 0.026	1.159 ± 0.066	1.331 ± 0.097

	Cytokine Production by PBMC (pg/mL)				
	IL-1β	IL-2	IL-6	IL10	TNF-β
0 µg/mL	574.66 ± 117.76	421.70 ± 112.23	117.35 ± 57.00	680.50 ± 118.18	468.84 ± 89.63
100 µg/mL	562.34 ± 122.42	481.08 ± 113.29	216.80 ± 38.61	627.48 ± 129.80	491.02 ± 90.83

	Cytokine Production by Monocytes (pg/mL)	
	IL-1α	TNF-α
0 µg/mL	381.08 ± 16.50	1061.94 ± 89.94
100 µg/mL	376.52 ± 28.73	1060.92 ± 58.45

	Basal Natural Killer Cell Activity (% Lysis)		
	100:1 E:T Ratio	33:1 E:T Ratio	11:1 E:T Ratio
0 µg/mL	59.56 ± 8.09	37.15 ± 11.18	15.84 ± 6.32
100 µg/mL	41.90 ± 6.37	35.47 ± 14.21	20.85 ± 14.60
	IL-2 Augmented Natural Killer Cell Activity (%Lysis)		
	100:1 E:T Ratio	33:1 E:T Ratio	11:1 E:T Ratio
0 µg/mL	77.66 ± 6.87	56.86 ± 10.14	28.97 ± 8.64
100 µg/mL	59.13 ± 4.45	48.73 ± 8.57	27.26 ± 11.65
	T Cell Mediated Cellular Cytotoxicity (%Lysis)		
	100:1 E:T Ratio	50:1 E:T Ratio	25:1 E:T Ratio
0 µg/mL	43.59 ± 6.98	41.68 ± 4.63	26.43 ± 3.11
100 µg/mL	42.21 ± 10.18	32.20 ± 4.94	18.49 ± 3.70

[a] Highest tested dose of natalizumab

PMBC = peripheral blood mononuclear cells; OD = optical density; PHA = phytohemagglutinin; IL = interleukin; TNF = tumor necrosis factor; E:T Ratio = Effector:Target Ratio

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3B Secondary Pharmacodynamics

Overview: Ex Vivo Immune Function Tests[a] **Test Article:** natalizumab

Study No. AL1106 **Location in CTD:** M4.2.3.2

Natalizumab Treatment Level	PBMC Basal Natural Killer Cell Activity (% Lysis)		
	100:1 E:T Ratio	33:1 E:T Ratio	11:1 E:T Ratio
0 mg/kg	30.24 ± 30.78	21.27 ± 23.96	12.42 ± 19.37
30 mg/kg[b]	34.29 ± 19.81	22.07 ± 10.06	11.19 ± 4.19
	Spleen Cell Basal Natural Killer Cell Activity (% Lysis)		
	100:1 E:T Ratio	33:1 E:T Ratio	11:1 E:T Ratio
0 mg/kg	35.07 ± 11.60	16.42 ± 7.88	6.79 ± 4.13
30 mg/kg	28.64 ± 14.23	15.89 ± 12.06	7.88 ± 9.34
	PHA Stimulated Proliferation of PBMC (OD at		
	1 µg/mL	2.5 µg/mL	5 µg/mL
0 mg/kg	0.083 ± 0.110	0.159 ± 0.157	0.246 ± 0.203
30 mg/kg	0.269 ± 0.169	0.339 ± 0.171	0.378 ± 0.139
	PHA Stimulated Proliferation of Spleen Cells (OD at		
	1 µg/mL	2.5 µg/mL	5 µg/mL
0 mg/kg	0.048 ± 0.057	0.101 ± 0.124	0.147 ± 0.140
30 mg/kg	0.192 ± 0.122	0.253 ± 0.126	0.289 ± 0.139
	LPS Stimulated Proliferation of PBMC (OD at		
	5 µg/mL	20 µg/mL	50 µg/mL
0 mg/kg	0.012 ± 0.024	0.025 ± 0.39	0.040 ± 0.084
30 mg/kg	0.030 ± 0.040	0.021 ± 0.024	0.035 ± 0.039
	LPS Stimulated Proliferation of Spleen Cells (OD at		
	5 µg/mL	20 µg/mL	50 µg/mL
0 mg/kg	0.053 ± 0.074	0.059 ± 0.066	0.057 ± 0.060
30 mg/kg	0.051 ± 0.045	0.069 ± 0.057	0.092 ± 0.086

[a] Measured at Day 28

[b] Cynomolgus monkeys, dosing Days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27

PMBC = peripheral blood mononuclear cells; OD = optical density; PHA = phytohemagglutinin; LPS = lipopolysaccharide; E:T Ratio = Effector:Target Ratio

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4 **Safety Pharmacology**

Test Article: natalizumab

Organ Systems Evaluated	Species/ Strain	Method of Admin.	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Cardiovascular/ Respiratory	Beagle Dog	IV	0.3, 3.0, 30.0[a]	3F	Transient decreases in blood pressure, contractile indices, cardiac output and stroke volume with variable (some increased some decreased) heart rate and peripheral resistance in 3 dogs (1 - 3 mg/kg, 2 - 30 mg/kg). No ECG or respiratory changes.	Yes	AL107 M4.2.1.3
Cardiovascular	Cynomolgus monkey	IV	0, 3.0, 10.0, 30.0, 60.0[b]	5F/5M (0, 60) 3F/3M (3, 10, 30)	No ECG, heart rate or blood pressure findings	Yes	723-013-98 M4.2.3.2
Cardiovascular	Cynomolgus monkey - juvenile	IV	0, 10.0, 30.0, 60.0[c]	5F/5M (0, 60) 3F/3M (10, 30)	No ECG, heart rate or blood pressure findings	Yes	309-011-00 M4.2.3.2
Cardiovascular	Rhesus Monkey	natalizumab – IV Avonex® - IM	Natalizumab[d] – 0, 30, 60 Avonex® – 0, 30 µg	3F/3M	No ECG, heart rate or blood pressure findings	Yes	P00002-01-01 M4.2.3.2

Additional Information:

[a]Single dose cross-over, sequenced as 30 minute infusion of vehicle, minimum 30 minute break, 30 minute infusion of natalizumab

[b]Multiple dose - weekly, cardiovascular testing at prestudy, Day 1 following the end of infusion, Week 4, 13, and 25

[c]Multiple dose - weekly, cardiovascular testing at prestudy, Day 1, 22, 85, 169 following the end of infusion

[d]Dosing was with natalizumab alone, Avonex® alone, or natalizumab in combination with Avonex®

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5 **Pharmacodynamic Drug Interactions**

Report Title: Avonex®/Antegren: A Four-Week Combination Toxicity Study of Antegren Administered Intravenously and Avonex® Administered Intramuscularly in the Rhesus Monkey Followed by an Eight-Week Recovery

Test Article: natalizumab

Species/Strain: Monkey/Rhesus
Initial Age: Prepubertal to young adult
Date of First Dose: July 9, 2001
Duration of Dosing: 4 weeks
Method of Administration: IV (natalizumab)/IM (Avonex®)
Vehicle/Formulation: Aqueous Solution
Study No. P00002-01-01
Location in CTD: M4.2.3.2
GLP Compliance: Yes

Dose (weekly): natalizumab (mg/kg):Avonex® (µg)	0:0	0:30	30:0	60:0	60:30	30:30
	M:F	M:F	M:F	M:F	M:F	M:F
Number of Animals	5:5	5:5	5:5	5:5	5:5	5:5
Avonex® Anti-viral Cytopathic Effect (U/mL)						
Day 1 Post-dose	0 ± 0	1266 ± 663	0 ± 0	100 ± 316[c]	660 ± 721	1140 ± 834
Day 8 Post-dose	170 ± 537[a]	1217 ± 653	0 ± 0	0 ± 0	886 ± 559	1230 ± 782
Day 15 Post-dose	0 ± 0	1297 ± 745	0 ± 0	0 ± 0	1233 ± 903	1158 ± 814
Day 22 Post-dose	0 ± 0	1373 ± 868	2 ± 5[b]	1 ± 3[d]	1653 ± 850	1173 ± 608
Natalizumab PBMC α4 Saturation (%)						
Day 22	NA	NA	96.8 ± 8.6	93.5 ± 4.6	99.2 ± 5.4	104.4 ± 15.3
Day 29	NA	NA	34.7 ± 52.5	54.2 ± 51.2	62.0 ± 33.6	34.0 ± 39.9
Day 56	NA	NA	8.8 ± 7.9	22.7 ± 33.1	24.5 ± 34.6	21.2 ± 24.9
Day 84	NA	NA	3.3 ± 0.6	12.8 ± 17.9	5.5 ± 3.5	4.2 ± 2.3

APPENDIX 4 – TABULATED SUMMARY OF PRECLINICAL PHARMACOKINETIC STUDIES CONDUCTED IN SUPPORT OF NATALIZUMAB

The following tables were copied directly from Module 2, section 2.6.3 of the electronic CTD submission, as provided by the sponsor to the BLA application.

1 Pharmacokinetics

Overview **Test Article:** natalizumab

Type of Study	Test System	GMP Drug Lot Number	Method of Administration	GLP Compliance	Testing Facility	Study Number	Location Section
Absorbtion (Kinetics)							
Pharmacokinetics in the Nude and SCID mouse – single and repeat dose	Nude mouse SCID mouse	F23014	IV, IP	No	Biogen	PD03-09	M4.2.2.2
Pharmacokinetics in the guinea pig	Guinea pig	NA[a] 10591	IC	No	Elan Pharmaceuticals	AL077	M4.2.2.2
Pharmacokinetics in the breeder guinea pig	Guinea pig	F23001 F23007	IV	Yes		309-010-01	M4.2.2.2
Pharmacokinetics in the monkey	Cynomolgus monkey	10591	IV	Yes/No[b]		AL109	M4.2.2.2
Pharmacokinetics in the monkey – formulation change	Cynomolgus monkey	10941	IV	Yes/No[b]		AL300	M4.2.2.2
Pharmacokinetics in the monkey – formulation change	Cynomolgus monkey	10941	IV	Yes/No[b]		723-012-98	M4.2.2.2
Comparison of 200L and — processes	Cynomolgus monkey	B81002A C0237	IV	Yes		723-004-98	M4.2.2.2
Comparison of — Biogen — processes	Cynomolgus monkey	MFG-133-01-04 E23001	IV	Yes		309-003-01	M4.2.2.2

1 **Pharmacokinetics (Continued)**

Type of Study	Test System	GMP Drug Lot Number	Method of Administration	GLP Compliance	Testing Facility	Study Number	Location Section
Comparison of — and — processes	Cynomolgus monkey	MFG-55-02-79 F23014	IV	Yes		P00002-02-01	M4.2.2.2
Distribution							
Placental transfer	Guinea pig	D23001	IV	Yes		309-025-00	M4.2.3.5.1
Placental transfer	Guinea pig	F23007	IV	Yes		309-028-02	M4.2.3.5.2
Placental transfer	Cynomolgus monkey	D23001	IV	Yes		309-012-00	M4.2.3.5.2
Breast milk and placental transfer	Cynomolgus monkey	F23007	IV	Yes		309-033-01	M4.2.3.5.3
Pharmacokinetic Drug Interactions							
Avonex®/natalizumab	Rhesus monkeys	MFG-113-01-31	Avonex® - SC natalizumab - IV	Yes		P00002-01-01	M4.2.3.2

[a] NA = Not Applicable, Research grade material

[b] Conducted as GLP, but considered to be non-GLP and suppportive only due to consent decree of April 2002 against

2 Analytical Methods And Validation Reports

Test Article: natalizumab

Assay Analyte	Assay Format	SOP Number	Testing Facility	Studies	Assay Procedure Location	Validation Report Location
					Section	Section
Natalizumab	Fluorometric ELISA	AAM-001-00102	Elan	AL105, AL106, AL109, AL300, AL302, 723-013-98, 723-004-98, 723-012-98, 309-011-00, 309-012-00, 309-003-01, P00002-01-01, 309-025-00	M4.2.2.1.2	M4.2.2.1.2
Anti-natalizumab Antibody	Fluorometric ELISA	AAM 001-00103	Elan	AL105, AL106, AL109, AL300, AL302, 723-013-98, 723-004-98, 723-012-98, 309-011-00, 309-012-00, 309-003-01, P00002-01-01	M4.2.2.1.3	M4.2.2.1.3
Anti-natalizumab Idiotype Antibody	Fluorometric ELISA	AAM 001-00322	Elan	723-004-98	M4.2.2.1.3	M4.2.2.1.3
Natalizumab	Colorimetric ELISA	GLP 001-00979	Elan	309-010-01, 309-005-02, 309-007-01	M4.2.2.1.2	M4.2.2.1.2
Anti-natalizumab Antibody	Colorimetric ELISA	GLP 001-00972	Elan	309-010-01, 309-005-02, 309-007-01	M4.2.2.1.3	M4.2.2.1.3
α4 Integrin Receptor Binding	FACS	AAM 001-00687	Elan	723-013-98, 309-011-00, P00002-02-01	M4.2.2.1.4	M4.2.2.1.4

2 Analytical Methods And Validation Reports (Continued)

Assay Analyte	Assay Format	SOP Number	Testing Facility	Studies	Assay Procedure Location	Validation Report Location
					Section	Section
Natalizumab Conc.	Colorimetric ELISA	SOP 097	Biogen Idec	P00002-02-01, PD03-09, P00002-03-01, P00002-03-04, 309-008-01, 309-009-01, 309-028-02, 309-033-01	M4.2.2.1.2	M4.2.2.1.2
Anti-natalizumab Antibody	Colorimetric ELISA	SOP 091	Biogen Idec	P00002-02-01, 309-008-01, 309-009-01, 309-028-02, 309-033-01	M4.2.2.1.3	M4.2.2.1.3
Avonex®	—	SOP 204	Biogen Idec	P00002-01-01	M4.2.2.1.5	M4.2.2.1.5
Anti-Avonex® Antibody	Colorimetric ELISA	SOP 203	Biogen Idec	P00002-01-01	M4.2.2.1.6	M4.2.2.1.6
Neutralizing Anti-Avonex® Antibody	Colorimetric ELISA	SOP 205	Biogen Idec	P00002-01-01	M4.2.2.1.6	M4.2.2.1.6
ELISA = Enzyme-linked Immunosorbent Assay, FACS = Fluorescence activated cell sorter, CPE = Cytopathic Effect						

3 Pharmacokinetics: Kinetics after a Single Dose

Test Article: natalizumab

Species	Nude Mouse	Hartley Guinea Pigs								
Study Number	PD03-09	AL077	309-010-01							
Location in CTD	M4.2.2.2	M4.2.2.2	M4.2.2.2							
Gender (M:F)/Number of Animals	0:96[b]	5:4	6:0	6:0	6:0	0:6	0:6	0:6	0:6	0:6	0:6
Special Conditions in Study	None	None	Male			Early Gestation[c]			Late Gestation[d]		
Vehicle/Formulation[a]	5	6	5								
Method of Administration	IV	IC	IV								
Sample	Serum	Serum	Serum								
Analyte	Natalizumab	Natalizumab	Natalizumab								
Assay	ELISA	ELISA	ELISA								
Dose (mg/kg)	10	3	3	10	30	3	10	30	3	10	30
PK Parameters:											
Cmax (µg/mL)	170	70 ± 10	72 ± 46	213 ± 141	700 ± 392	53 ± 27	292 ± 50	532 ± 254	59 ± 34	258 ± 91	629 ± 517
AUC (µg*hr/mL)	11728	1504 ± 406	1694 ± 988	4458 ± 2489	17457 ± 4748	1424 ± 440	7290 ± 1320	16804 ± 6453	1505 ± 692	6169 ± 1721	15104 ± 7500
(Time for calculation – hr)	240	168	48								
$T_{1/2}$ (hr)	77	36.9	NR	NR	NR	NR	NR	NR	NR	NR	NR
(Time for calculation – hr)	240	168	48								

[a] Vehicle/Formulation 5 = 5 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0
Vehicle/Formulation 6 = 3 or 4.2 mg/mL Natalizumab in PBS (two drug lots used for the study)

[b] Sacrificed in groups of three per TK time point

[c] Early Gestation = GD4 at study initiation

[d] Late Gestation = GD30 at study initiation

NR = Not reported

3 Pharmacokinetics: Kinetics after a Single Dose (continued)

Species	Cynomolgus Monkeys								
Study Number	AL109	AL300		723-012-98		723-004-98		309-003-01	
Location in CTD	M4.2.2.2	M4.2.2.2		M4.2.2.2		M4.2.2.2		M4.2.2.2	
Gender (M:F)/Number of Animals	4:4	0:3		0:3		0:8		0:15	
Special Conditions in Study	None	None		None		—		—	Biogen
Vehicle/Formulation[a]	1	1	2	1	3	4	3	5	5
Method of Administration	IV	IV		IV		IV		IV	
Sample	Serum	Serum		Serum		Serum		Serum	
Analyte	Natalizumab	Natalizumab		Natalizumab		Natalizumab		Natalizumab	
Assay	ELISA	ELISA		ELISA		ELISA		ELISA	
Dose (mg/kg)	3	3		3		3		3	
PK Parameters:									
Cmax (µg/mL)	124 ± 35	79 ± 30	58 ± 14	62 ± 17	43 ± 3	67 ± 7	64 ± 5	85 ± 16	87 ± 15
AUC (µg*hr/mL)	2537 ± 628	1962 ± 648	1729 ± 601	1666 ± 165	1332 ± 303	1217 ± 137	1120 ± 103	2307 ± 566	1948 ± 570
(Time for calculation – hr)	672	672		672		672		672	
$T_{1/2}$ (hr)	58	80	65	64	60	68	62	88	99
(Time for calculation – hr)	672	672		672		672		672	

[a] Vehicle/Formulation 1 = 5 mg/mL Natalizumab in 50 mM Histidine, 150 mM NaCl, pH 6.0
Vehicle/Formulation 2 = 5 mg/mL Natalizumab in 50 mM Histidine, 150 mM NaCl, 0.02% PS80, pH 6.0
Vehicle/Formulation 3 = 5 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0
Vehicle/Formulation 4 = 1.7 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0
Vehicle/Formulation 5 = 20 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0

3 Pharmacokinetics: Kinetics after a Single Dose (continued)

Species	Cynomolgus Monkeys								
Study Number	P00002-02-01		723-013-98				309-011-00		
Location in CTD	M4.2.2.2		M4.2.3.2				M4.2.3.2		
Gender (M:F)/Number of Animals	0:8	0:8	3:3	3:3	3:3	5:5	3:3	3:3	5:4
Special Conditions in Study	[redacted]		None				Juveniles	Juveniles	Juveniles
Vehicle/Formulation*	5		3				5		
Method of Administration	IV		IV				IV		
Sample	Serum		Serum				Serum		
Analyte	Natalizumab		Natalizumab				Natalizumab		
Assay	ELISA		ELISA				ELISA		
Dose (mg/kg)	3		3	10	30	60	10	30	60
PK Parameters:									
Cmax (µg/mL)	74 ± 24	74 ± 13	48 ± 6	184 ± 40	690 ± 144	1787 ± 355	172 ± 67	492 ± 133	1168 ± 311
AUC (µg*hr/mL)	3574 ± 939	3894 ± 945	1391 ± 574	7444 ± 3462	36281 ± 21406	141221 ± 47262	11340 ± 3353	55264 ± 17621	143057 ± 76479
(Time for calculation – hr)	408		168				168		
$T_{1/2}$ (hr)	67	61	36	37	50	69	71	115	127
(Time for calculation – hr)	408		168				168		

* Vehicle/Formulation 3 = 5 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0
Vehicle/Formulation 5 = 20 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0

4 Pharmacokinetics: Kinetics after Repeated Doses

Species	Hartley Guinea Pig								
Study Number	309-010-01								
Location in CTD	M4.2.2.2								
Gender (M/F)/Number of Animals	6:0			0:6			0:6		
Special Conditions in Study	Male			Early Gestation			Late Gestation		
Vehicle/Formulation*	5								
Method of Administration	IV								
Sample	Serum								
Analyte	Natalizumab								
Assay	ELISA								
Dose (mg/kg)	3	10	30	3	10	30	3	10	30
PK Parameters:									
Peak Cmax (µg/mL)	117 ± 41	974 ± 351	2035 ± 719	124 ± 44	577 ± 120	1072 ± 727	157 ± 38	918 ± 331	1875 ± 383
AUC (µg x hr/mL): Cumulative	34048 ± 19465	567178 ± 330879	1556793 ± 124112	23043 ± 4844	131574 ± 46128	577012 ± 224675	98109 ± 34485	522124 ± 276162	1641053 ± 461496
(Time for calculation – days)	67	67	67	26	26	26	66	66	66

* Vehicle/Formulation 5 = 20 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0

4 Pharmacokinetics: Kinetics after Repeated Doses (continued)

Species	Cynomolgus Monkey						
Study Number	723-013-98				309-011-00		
Location in CTD	M4.2.3.2				M4.2.3.2		
Gender (M/F)/Number of Animals	3:3	3:3	5:5	5:5	3:3	3:3	5:4
Special Conditions in Study	None				Juvenile		
Vehicle/Formulation*	3				5		
Method of Administration	IV				IV		
Sample	Serum				Serum		
Analyte	Natalizumab				Natalizumab		
Assay	ELISA				ELISA		
Dose (mg/kg)	3	10	30	60	10	30	60
PK Parameters:							
Cmax (µg/mL)	60 ± 9	272 ± 42	1042 ± 392	2654 ± 1143	244 ± 36	952 ± 344	2470 ± 476
AUC (µg x hr/mL): Cumulative	6529 ± 616	31978 ± 11	1049836 ± 1181815	4461260 ± 3884654	172660 ± 143870	1814932 ± 1418766	6527648 ± 1697479
(Time for calculation – months)	6	6	6	6	6	6	6

* Vehicle/Formulation 3 = 5 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0
Vehicle/Formulation 5 = 20 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0

4 **Pharmacokinetics: Kinetics after Repeated Doses (continued)**

Species	Cynomolgus Monkey							
Study Number	309-012-00						309-033-01	
Location in CTD	M4.2.3.5.2						M4.2.3.5.3	
Gender (M/F)/Number of Animals	9		8		13		11	15
Special Conditions in Study	Pregnant						Pregnant	
Vehicle/Formulation[a]	5						5	
Method of Administration	IV						IV	
Sample	Serum						Serum	
Analyte	Natalizumab						Natalizumab	
Assay	ELISA						ELISA	
Dose (mg/kg)	3		10		30		30 (GD20-70)	30 (GD20-Term)
PK Parameters:								
Peak Cmax (µg/mL)	78 ± 16		3366 ± 2361		13487 ± 7993		4122 ± 1452	6220 ± 3212
AUC (µg x hr/mL): Cumulative	22265 ± 4585	23134 ± 6422	469657 ± 308855	1660532 ± 1100144	2926875 ± 2191711	8690898 ± 5251820	NA	NA
(Time for calculation – days)	24	80	24	80	24	80	150	150

[a] Vehicle/Formulation 5 = 20 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0

NA = Not applicable, analysis of these parameters not yet available for this study.

5 Pharmacokinetics: Organ Distribution

Studies of organ distribution (e.g., radiolabeled distribution) were not performed for natalizumab. Evaluation of the distribution of the target of natalizumab, α4 integrins, was performed via standard tissue cross-reactivity studies. These studies are reported in Section 8 of Report 2.6.6, Toxicology Written Summary.

6 Pharmacokinetics: Plasma Protein Binding

Not applicable. Monoclonal antibodies do not bind other plasma proteins unless specifically developed to target those proteins. The target of natalizumab is a cell surface, membrane bound protein that does not exist free in the plasma.

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7A Pharmacokinetics: Study In Pregnant Or Nursing Animals

Test Article: Natalizumab
Location in CTD: M4.2.3.5.1
Study Number: 309-025-00

Placental Transfer

Species:	Hartley Guinea Pigs
Vehicle/Formulation:	5[a]
Method of Administration:	IV, every-other-day
Analyte:	Natalizumab
Assay:	ELISA
Day for Analysis:	GD29
Number of Animals for Analysis	14
Dosing Period:	GD4-GD28
Dose (mg/kg):	30
Mean Concentration/Amount	
Maternal Serum (µg/mL)	813.8 ± 679.7
Fetus/Neonate (µg/mL)	4.9 ± 4.1
Fetal/Maternal Ratio	0.007
[a] Vehicle/Formulation 5 = 20 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0	

7B Pharmacokinetics: Study in Pregnant or Nursing Animals

Test Article: Natalizumab
Location in CTD: M4.2.3.5.2
Study Number: 309-028-02

Placental Transfer

Species:	Hartley Guinea Pigs
Vehicle/Formulation:	5[a]
Method of Administration:	IV, every-other-day
Analyte:	Natalizumab
Assay:	ELISA
Day for Analysis:	GD60 of second pregnancy
Number of Animals for Analysis	29
Dosing Period:	GD30 of first pregnancy to GD30 of second pregnancy (48-99 days, mean 63 days)
Dose (mg/kg):	30
Mean Concentration/Amount	
Maternal Serum (µg/mL)	6.3
Fetus/Neonate (µg/mL)	8.7
Fetal/Maternal Ratio	3.354
[a] Vehicle/Formulation 5 = 20 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0	

7C Pharmacokinetics: Study in Pregnant or Nursing Animals

Test Article: Natalizumab
Location in CTD: 4.2.3.5.2
Study Number: 309-012-00

Placental Transfer

Species:	Cynomolgus Monkeys		
Vehicle/Formulation:	5[a]		
Method of Administration:	IV, every-other-day		
Analyte:	Natalizumab		
Assay:	ELISA		
Day for Analysis:	GD100		
Number of Animals for Analysis	9	8	13
Dosing Period:	GD20-GD70		
Dose (mg/kg):	3	10	30
Mean Concentration/Amount			
Maternal Serum (µg/mL)	0	27 ± 17	170 ± 131
Fetus/Neonate (µg/mL)	0	7 ± 10	69 ± 69
Fetal/Maternal Ratio	NA	0.180	0.348

[a] Vehicle/Formulation 5 = 20 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0
NA = Not applicable

7D Pharmacokinetics: Study in Pregnant or Nursing Animals

Test Article: Natalizumab
Location in CTD: M4.2.3.5.3
Study Number: 309-033-01

Placental Transfer

Species:	Cynomolgus Monkeys		
Vehicle/Formulation:	5[a]		
Method of Administration:	IV, every-other-day		
Analyte:	Natalizumab		
Assay:	ELISA		
Day for Analysis:	PND28		
Number of Animals for Analysis	5	7	
Dosing Period:	GD20-GD70	GD20-Term	
Dose (mg/kg):	30	30	
Mean Concentration/Amount			
Maternal Serum (µg/mL)	0.40[b]	412 ± 399	
Fetus/Neonate (µg/mL)	0.46[b]	348 ± 286	
Fetal/Maternal Ratio	1.15[b]	1.89	

[a] Vehicle/Formulation 5 = 20 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0
[b] Results are for the single dam/infant pair at this dose level where both infant and dam had measurable serum natalizumab levels. For the other four pairs of animals both the dam and the infant had no measurable serum natalizumab levels - so no ratio could be calculated.

7E Pharmacokinetics: Study in Pregnant or Nursing Animals

Test Article: Natalizumab
Location in CTD: M4.2.3.5.5
Study Number: 309-033-01

Excretion in Milk

Species:	Cynomolgus Monkeys		
Vehicle/Formulation:	5[a]		
Method of Administration:	IV, every-other-day		
Analyte:	Natalizumab		
Assay:	ELISA		
Day for Analysis:	PND28		
Number of Animals for Analysis		5	7[b]
Dosing Period:		GD20-GD70	GD20-Term
Dose (mg/kg):		30	30
Mean Concentration/Amount			
Maternal Serum (µg/mL)		0.0	412 ± 399
Maternal Milk (µg/mL)		0.0	0.00
Milk/Serum Ratio		NA	0.00

[a] Vehicle/Formulation 5 = 20 mg/mL Natalizumab in 10 mM Sodium Phosphate, 140 mM NaCl, 0.02% PS80, pH 6.0

[b] Only 1/7 dams were observed to have both serum and milk with measurable natalizumab levels. For this single animal the Milk/Serum ratio was 0.004. For the other six animals measurable serum natalizumab levels (ranging from [redacted]) were found in the serum but milk levels were 0.0.

Appears This Way
On Original

8 Other Distribution Studies

Not applicable. No other studies of distribution were performed for natalizumab.

9 Pharmacokinetics: Metabolism In Vivo

Not applicable per ICH S6. Metabolism of natalizumab, like all antibodies, will occur via normal proteolytic processes to individual amino acids through cleavage by exopeptidases and endopeptidases. As these pathways are generally understood, studies specific to natalizumab are not needed.

10 Pharmacokinetics: Metabolism In Vitro

Not applicable per ICH S6. Metabolism of natalizumab, like all antibodies, will occur via normal proteolytic processes to individual amino acids through cleavage by exopeptidases and endopeptidases. As these pathways are generally understood, studies specific to natalizumab are not needed.

11 Pharmacokinetics: Possible Metabolic Pathways

Not applicable per ICH S6. Metabolism of natalizumab, like all antibodies, will occur via normal proteolytic processes to individual amino acids through cleavage by exopeptidases and endopeptidases. As these pathways are generally understood, studies specific to natalizumab are not needed.

12 Pharmacokinetics: Induction/Inhibition of Drug-Metabolizing Enzymes

Not applicable per ICH S6. Metabolism of natalizumab, like all antibodies, will occur via normal proteolytic processes to individual amino acids through cleavage by exopeptidases and endopeptidases. As these pathways are generally understood, studies specific to natalizumab are not needed.

13 Pharmacokinetics: Excretion

Not applicable per ICH S6. The free amino acids formed by proteolysis of natalizumab will be subject to normal intermediate metabolism, biosynthetic processes, and excretion. As these pathways are generally understood, studies specific to natalizumab are not needed

14 Pharmacokinetics: Excretion into Bile

Not applicable per ICH S6. The free amino acids formed by proteolysis of natalizumab will be subject to normal intermediate metabolism, biosynthetic processes, and excretion. As these pathways are generally understood, studies specific to natalizumab are not needed

15 Pharmacokinetics: Drug-Drug Interaction

Test Article: Natalizumab
Location in CTD: M4.2.3.2
Study Number: P00002-01-01

Type of Study:	A 28-day combination toxicity study of natalizumab and Avonex® was performed in rhesus monkeys. This study incorporated measures of natalizumab and Avonex® serum concentrations, antibodies to natalizumab and Avonex®, natalizumab-induced hematology changes, and α4 integrin receptor saturation by natalizumab.
Method:	Avonex® serum concentration: Indirect measurement based on anti-viral activity against EMC virus Natalizumab serum concentration: Colorometric ELISA Anti-Avonex® serum antibody concentration: Colorometric ELISA Anti-Natalizumab serum antibody concentration: Colorometric ELISA Natalizumab-induced hematology changes: Advia automated hematology analyzer Natalizumab α4 integrin receptor saturation: FACS analysis of lymphocytes
Tabulated Results:	Avonex® serum concentration: Unchanged in combination treatment groups. Natalizumab serum concentration: Unchanged in combination treatment groups. Anti-Avonex® serum antibody concentration: Unchanged in combination treatment groups. Anti-Natalizumab serum antibody concentration: Unchanged in combination treatment groups. Natalizumab-induced hematology changes: Unchanged in combination treatment groups. Natalizumab α4 integrin receptor saturation: Unchanged in combination treatment groups.
Additional Information: Animals were treated weekly for four weeks with Vehicle, 30 mg/kg of natalizumab, 60 mg/kg of natalizumab, 30 µg/dose of Avonex®, 30 mg/kg of natalizumab + 30 µg/dose of Avonex®, or 60 mg/kg of natalizumab + 30 µg/dose of Avonex®	

16 Pharmacokinetics: Other

Not applicable. No additional pharmacokinetic studies of natalizumab were performed.