(b) (4)

FINAL REPORT

Ad26 (b) (4): 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Test Article:
Ad26 (b) (4)

Sponsor:
Beth Israel Deaconess Medical Center
Division of Viral Pathogenesis
Research East Room 213
41 Avenue Louis Pasteur
Boston, Massachusetts 02115

Testing Facility:
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Authors:
(b) (4), (b) (6)

Study Completion Date:
September 14, 2007

Document No.: (b) (4)

TABLE OF CONTENTS

COMPLIANCE STATEMENT

Ad26 (b) (4): 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

This study was conducted in compliance with current U.S. FDA Good Laboratory Practice (GLP) Regulations for Non-clinical Laboratory Studies (21 CFR Part 58) with the following exceptions:

- Stability analyses of the placebo/control article has not been provided by the Sponsor.
- Characterization of the test article was performed under GRP regulations.
- Characterization of the control article was performed under GMP regulations.

There were no deviations from the aforementioned regulations that affected the quality or integrity of the study or the interpretations of the results in this report.

Study Director:

(b) (4), (b) (6)

9-14-07
Date

QUALITY ASSURANCE STATEMENT

Ad26 (b) (4): 91-Day Intramuscular Single Dose
Biodistribution Study in New Zealand White Rabbits

This study was inspected/audited by Quality Assurance in accordance with (b) (4) (b) (4) Standard Operating Procedures, the protocol, and FDA Good Laboratory Practice regulations. All findings were reported to the Study Director and Testing Facility Management as indicated below.

		Date Reported	
Type of Audit	**Date(s) Audited**	**Study Director**	**Management**
Protocol Audit	January 30, 2007	January 31, 2007	January 31, 2007
Dose Administration	February 2, 2007	February 5, 2007	February 5, 2007
Draft Report and Raw Data	June 25-26, 2007	June 27, 2007	June 27, 2007
Final Report Post Audit	September 10, 2007	September 10, 2007	September 10, 2007

The Biodistribution analysis was conducted by (b) (4) under the purview of their QAU. (b) (4) Quality Assurance Statement is presented in Appendix No. 6.

Action has been taken in response to all items listed by Quality Assurance. It is concluded that the final report accurately reflects (b) (4) Standard Operating Procedures and the raw data for this study.

(b) (4), (b) (6)
Sr. Quality Assurance Auditor

14 Sept 2007
Date

SIGNATURE PAGE

Ad26 (b) (4): 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Authors:

(b) (4), (b) (6)

Study Director

9-14-07
Date

(b) (4), (b) (6)

Toxicology Associate

9/14/2007
Date

Peer Review:

(b) (4), (b) (6)

Senior Director of Toxicology

9/14/07
Date

SUMMARY

Ad26 (b) (4) 91-Day Intramuscular Single Dose
Biodistribution Study in New Zealand White Rabbits

The purpose of this study was to determine the biodistribution of an adenovector-based (b) (4) vaccine in New Zealand White rabbits during a 91-day study period when administered by a single intramuscular injection.

Forty eight rabbits were randomly assigned to one of two groups (9/sex in Group 1 and 15/sex in Group 2). Animals were administered placebo (Group 1) or Ad26 (b) (4) at 0.5 x 10^{11} virus particles (Group 2) via intramuscular injection on Study Day (SD) 1. Necropsies were performed on 3 animals/sex in Group 1 and 5 animals/sex in Group 2 on SD 11, 61, and 91 to collect tissues for biodistribution analysis. Parameters evaluated during the study included clinical and cageside observations, body weights, body weight changes, and biodistribution.

Treatment with Ad26 (b) (4) vaccine at a dose 0.5 x 10^{11} virus particles did not affect mortality, clinical observations or body weights.

Quantitative PCR (qPCR) analysis indicated that the vaccine was primarily localized in the spleen, iliac lymph node, and the muscle at the site of injection. By SD 61, the vaccine was no longer detected in the spleen. By SD 91, detection of the vector in the iliac lymph nodes and injection site muscle was noted in only 2 of 10 treated animals.

In conclusion, a single intramuscular injection of Ad26 (b) (4) vaccine (0.5 x 10^{11} virus particles) was well tolerated in male and female New Zealand White Rabbits. Biodistribution analysis indicated that Ad26 (b) (4) was cleared from most of the examined tissues by SD 91.

STUDY PERSONNEL AND TEST SITES

Study Director:	(b) (4), (b) (6)
Toxicology Associate:	(b) (4), (b) (6)
Report Associate:	(b) (4), (b) (6)
Technical Supervisor:	(b) (4), (b) (6)
Manager, Formulations:	(b) (4), (b) (6)
Supervisor, Necropsy:	(b) (4), (b) (6)
Director, Vivarium Operations:	(b) (4), (b) (6)
Director, Laboratory Animal Medicine:	(b) (4), (b) (6)
Sponsor:	Beth Israel Deaconess Medical Center Division of Viral Pathogenesis Research East Room 213 41 Avenue Louis Pasteur Boston, MA 02115
Sponsor's Representative:	(b) (4), (b) (6)
Laboratory Operations Animal Facility:	(b) (4)
Biodistribution Analysis:	(b) (4), (b) (6) (b) (4)
Archives Data:	(b) (4)
Preserved Specimens:	(b) (4)

STUDY TIMETABLE

Study Initiation Date:	January 8, 2007
Experimental Start Date/Receipt of Animals:	January 22, 2007
Randomization of Animals:	February 1, 2007
Dosing:	February 2, 2007
Necropsy	
Terminal (SD 11):	February 12, 2007
Recovery 1 (SD 61):	April 3, 2007
Recovery 2 (SD 91):	May 3, 2007
Experimental Completion Date:	July 6, 2007
Study Completion Date:	September 14, 2007

INTRODUCTION

The purpose of this study was to determine the biodistribution of an adenovector-based (b) (4) vaccine in New Zealand White rabbits during a 91-day study period when administered by a single intramuscular injection.

The rabbit was used for the study because of the FDA recommendation for assessing the biodistribution of adenovirus-vectored vaccines. The intramuscular route was selected since it is the intended route of human exposure. The dose for this study was selected based on the largest amount of vaccine that can be delivered into the rabbit muscle with one injection.

The protocol, amendments, and deviations are presented in Appendix 7.

METHODS AND MATERIALS

Test and Control Articles

Neat Materials

The neat test and control articles used on this study are described in Text Table 1.

Text Table 1: Neat Test and Control Articles

Name	Lot No.	Supplier	Purity	Description
Ad26 (b) (4) Placebo	06M05/01	Crucell Holland BV The Netherlands	Assumed 100%	Clear solution
Ad26 (b) (4)	06K02/01	Crucell Holland BV The Netherlands	Assumed 100%	Clear to slightly opalescence solution

The test article, Ad26 (b) (4) was received on dry ice and stored frozen at -75 ± 15°C upon receipt. The control article, Ad26 (b) (4) Placebo, was received on cool packs and stored refrigerated at 2 - 8 C upon receipt. The Certificates of Analysis are presented in Appendix 1.

No reserve samples were taken for this study because reserve samples of the same lot were taken in study (b) (4)

Any remaining test and control articles were returned to the Sponsor, and any empty containers were discarded on September 4, 2007.

Dose Formulations

No formulations were required because the control and test articles were supplied in ready-to-use form.

On the day of dosing, the appropriate number of control article vials were removed from the refrigerator and allowed to equilibrate to room temperature for approximately 30 minutes. The appropriate number of test article vials were removed from the freezer and allowed to thaw and equilibrate to room temperature for approximately 45 minutes.

Dosing materials, dispensed in pre-filled syringes, were maintained at room temperature before and during dosing and were used within 4 hours after thawing.

Stability analysis of the test article is presented in Appendix 1.

Test Animals and Husbandry

Animals

Animal information is provided in Text Table 2.

Text Table 2: Animal Information

	Males	Females
Species and Strain	New Zealand White rabbits (HsdOkd)	
Supplier	(b) (4)	
Number of Animals Received	26	26
Number Used on Study	24	24
Age at Receipt	11 – 16 weeks	11 – 16 weeks
Weight Range at Receipt	2276 – 2606 g	2100 – 2533 g
Disposition of Extra Animals	Extra animals were transferred to the training colony	

Animals were acclimated to laboratory conditions for at least 7 days prior to the first dose and released from acclimation by a staff veterinarian. During that time, animals were identified by a temporary number that was recorded on each cage label.

The Institutional Animal Care and Use Committee (IACUC) of (b) (4) approved this protocol and found it to be in accordance with provisions of the USDA Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals and the US Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals.

Husbandry

Animal husbandry was provided as described in Text Table 3.

Text Table 3: Husbandry Information

Feed	Certified Global Harlan Teklad Laboratory Diet 2030
Water	Filtered tap water via an automatic watering system and/or water bottles
Housing	Individually housed in cages suspended on stainless steel racks
Temperature Range	16 to 22°C
Humidity Range	30 to 70%
Light Cycle	12-hour light/12-hour dark, interrupted as necessary for study related events
Air Changes	Minimum of 10 air changes per hour

Feed and water were provided *ad libitum*, unless otherwise noted. The feed was analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. The water is routinely analyzed for contaminants and specific microbes. No contaminants were known to be present in the feed or water at levels that might have interfered with achieving the objectives of the study.

Environmental controls were set to maintain animal room conditions as shown in Text Table 3. Actual temperature and relative humidity in the animal room or zone were monitored continuously by a computerized system. All environmental parameters were maintained within the protocol requirements with the exception of deviations noted in Appendix 7. The deviations had no effect on the health of the animals and/or the outcome of the study.

All animals were provided jingle balls for environmental enrichment.

Experimental Design

Group Assignment and Doses

Animals were initially accepted into the randomization pool based upon body weights and physical examinations. They were assigned to study groups using computer-generated random numbers. At randomization the mean body weight for each group was not statistically different ($p<0.05$) from the control mean. The animals were assigned to groups as shown in Text Table 4.

Text Table 4: Study Design

Group	Treatment	Dose Level (virus particles)	Dose Volume (mL)	Total Number of Animals	Scheduled Sacrifice Timepoint		
					SD 11	SD 61	SD 91
1	Placebo	0	0.5	9/sex	3/sex	3/sex	3/sex
2	Ad26 (b) (4)	0.5×10^{11}	0.5	15/sex	5/sex	5/sex	5/sex

After randomization, each study animal was assigned a unique number and identified by ear tag. Animal assignment is presented in Text Table 5.

Text Table 5: Animal Assignment

Group	SD 11 Necropsy		SD 61 Necropsy		SD 91 Necropsy	
	Males	Females	Males	Females	Males	Females
1	14530-14532	14539-14541	14533-14535	14542-14544	14536-14538	14545-14547
2	14548-14552	14563-14567	14553-14557	14568-14572	14558-14562	14573-14577

Dose Administration

Dosing information is presented in Text Table 6.

Text Table 6: Dose Administration Information

Route of Administration	Intramuscular
Frequency of Dosing	Once on SD 1
Dose Volume	0.5 mL
Dose Sites	Right hind thigh muscle
Equipment	1-cc insulin syringe with a 27-gauge 5/8-inch needle
Dosing Conditions	Formulations were kept at room temperature during dosing

Each dose site was shaved and marked prior to dosing and all formulations were dosed within 4 hours of thawing.

Observations

Animals were observed as shown in Text Table 7.

Text Table 7: Animal Observations/Measurements

Procedure	Frequency of Testing
Cageside Observations	≥ Twice daily
Clinical Observations	Prior to each dose, weekly thereafter, and at termination
Body Weight	Prior to each dose, weekly thereafter, and at termination

Cageside observations included observation for mortality, moribundity, general health and signs of toxicity. Clinical observations included evaluation of skin and fur characteristics, injection site, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor and behavior patterns.

Termination, Necropsy and Tissue Collection

Blood Collection

Prior to termination, whole blood (≥ 0.6 mL) was collected into K_3 EDTA tubes via puncture of the medial auricular artery for biodistribution analysis. The tubes were inverted several times and the blood was transferred to cryovials, snap frozen in liquid nitrogen, and stored frozen at -75 ± 15°C.

Termination

On SD 11 (Terminal Kill), 61 (Recovery Kill 1), and 91 (Recovery Kill 2), three rabbits per sex from Group 1 and five rabbits per sex from Group 2 were euthanized by intravenous injection of sodium pentobarbital and exsanguinated.

Necropsy

Animals were necropsied as soon as possible after euthanasia. A gross necropsy, which included examination of the external surface of the body, injection site, all orifices, and the cranial, thoracic, and abdominal cavities and their contents, was performed.

Tissue Collection

Group 1 (control) animals were necropsied first, followed by Group 2 animals. The following tissues were collected: ovaries/testes, liver (left lateral), thymus, heart (apex), lung (right diaphragmatic lobe), kidney (hilar region), spleen (median region), mesenteric lymph nodes, iliac lymph nodes, skin and subcutis at injection site, thigh muscle at injection site, bone marrow from left femur, and brain. Paired organs were processed together. A fresh set of sterile instruments and a new pair of gloves were used for each organ of each animal. All tissues were snap frozen in liquid nitrogen and stored at -75 ± 15°C.

All specimens collected, including the whole blood, were shipped (on dry ice) to (b) (4) following each necropsy. All tissues collected on SD 11, 61, and 91 were processed and analyzed for the presence of the Ad26 (b) (4) using a qualified quantitative polymerase chain reaction (qPCR) method. The Biodistribution Report is presented in Appendix 6.

Data Collection and Statistical Analyses

Electronic data collection, including dosing, animal husbandry and environmental enrichment, clinical observations, body weight, and body weight change, was performed using Provantis™ NT 2000 (b) (4) (b) (4)

Body weights and body weight changes were analyzed using the Kolmogorov-Smirnov test for normality, the Levene Median test for equal variance, and by one-way Analysis of Variance (ANOVA). If either the normality or equal variance test failed, then the analysis was continued using the non-parametric Kruskal-Wallis ANOVA on rank-transformed data. For parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunnett's t-test was used to delineate which groups (if any) differed from the control. For non-parametric data, if the ANOVA indicated statistical significance among experimental groups then the Dunn's test was used to delineate which groups (if any) differed from the control. The probability value of less than 0.05 (two-tailed) was used as the critical level of significance for all tests.

Statistical analysis was conducted using SigmaStat™ Statistical Software, Version 1 (b) (4) (b) (4) The term "significant" is used throughout the text of the report to indicate statistical significance at $p<0.05$.

Record Retention

All study data, including but not limited to, animal data, formulations data, necropsy data, professional reports, study protocol (including amendments), final report and any communications concerning the conduct of the study will be retained in the archive of (b) (4) for a period of 5 years following completion of the final report. Due to a presumed limited stability, preserved tissues will be maintained for a 1-year period at (b) (4)

Following the 5-year period (or before at Sponsor's request), the Sponsor will be contacted to determine the disposition of these materials. All electronic data will be maintained at (b) (4) Records regarding disposition of data and specimens will be maintained at (b) (4) Study data generated by the Sponsor or sub-contractors will be archived by the Sponsor or sub-contractors, respectively.

RESULTS

Stability Analysis

As shown in the Stability Reports presented in Appendix 1, Ad26 (b) (4) is considered stable at room temperature for at least 4 hours, and at accelerated stability storage condition (2-8°C) for at least 6 months.

Animal Disposition and Observations

Data are presented in Table 1 (animal disposition and clinical observations) and Table 2 (cageside observations). Individual data are presented in Appendix 3.

A single intramuscular injection of Ad26 (b) (4) vaccine had no effect on mortality or clinical/cageside observations. All animals survived until the scheduled termination. The only incidental clinical observation noted was a urine stain for one Group 2 male on SD 91.

Body Weight and Body Weight Changes

Data are summarized in Table 3 (body weights) and Table 4 (body weight changes). Mean body weights are presented graphically in Figure 1 (males) and Figure 2 (females). Individual data are presented in Appendix 4 and Appendix 5, respectively.

Figure 1: Mean Body Weights – Males

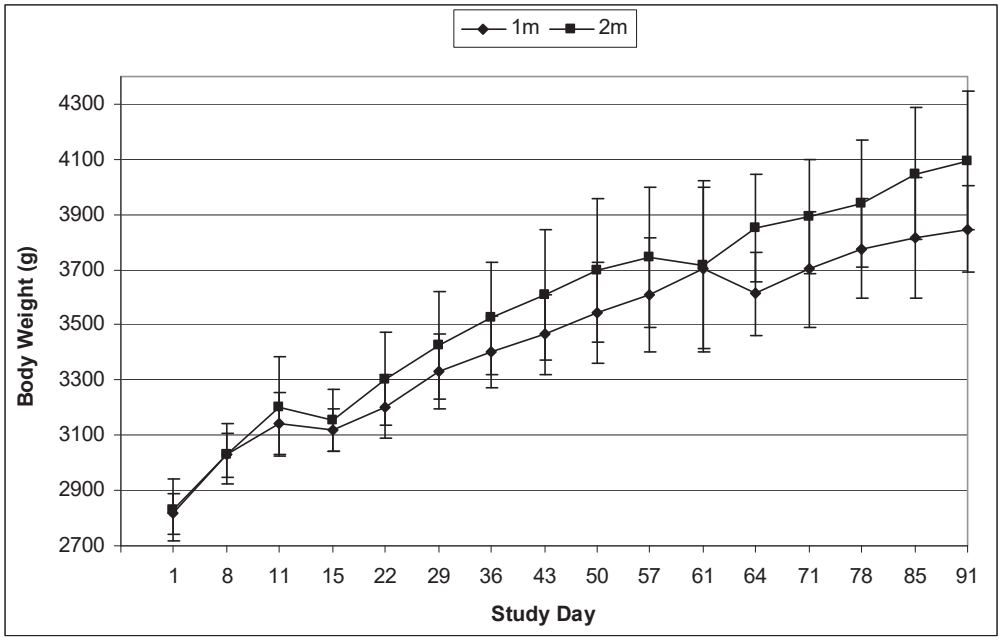
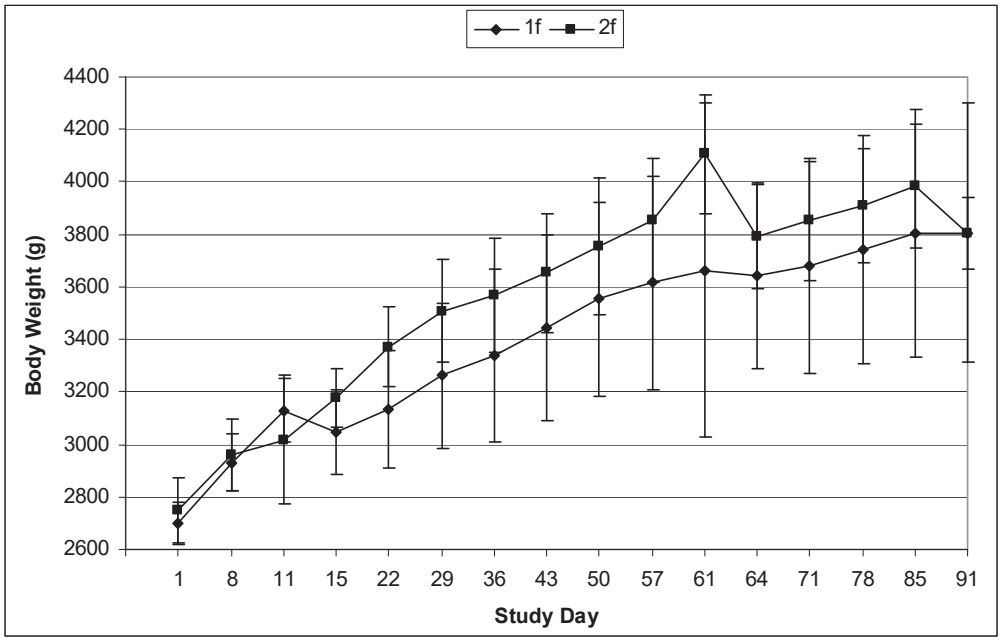


Figure 2: Mean Body Weights – Females



A single intramuscular injection of Ad26 (b) (4) vaccine had no effect on body weight or body weight changes. There were a few incidental, sporadic statistically significant differences noted in the body weight and body weight change data; the findings were not considered to be biologically or toxicologically significant.

Biodistribution Analysis

The Biodistribution Report is presented in Appendix 6.

Quantitative PCR (qPCR) analysis indicated that the vaccine was primarily localized in the spleen, iliac lymph nodes, and the muscle at the site of injection. By SD 61, the vaccine was no longer detected in the spleen. By SD 91, detection of the vector in the iliac lymph nodes and injection site muscle was limited to 2 of 10 treated animals.

CONCLUSION

A single intramuscular injection of Ad26 (b) (4) vaccine (0.5 x 10^{11} virus particles) was well tolerated in male and female New Zealand White rabbit. Biodistribution analysis indicated that Ad26 (b) (4) was cleared from almost all examined tissues by SD 91. Detection of the vector was limited to the iliac lymph nodes and injection site muscle in 2 of 10 treated animals.

ABBREVIATIONS

Not all abbreviations listed are used in this report.

↑	greater than control	**S.D.**	standard deviation
↓	less than control	**RSD**	relative standard deviation
>	greater than	**TK**	toxicokinetic
<	less than	**PK**	pharmacokinetic
≥	greater than or equal to	**AUC**	area under the curve
≤	less than or equal to	**C_{max}**	maximum concentration
~	approximately	**$t_{1/2}$**	half-life
°	degree	**SD**	study day
%	percent	**GD**	gestation day
C	Celsius	**PND**	post-natal day
F	Fahrenheit	**i.p.**	intraperitoneal
L	liter	**i.v.**	intravenous
mL	milliliter	**s.c.**	subcutaneous
µL	microliter	**i.m.**	intramuscular
g	gram	**EPA**	Environmental Protection Agency
kg	kilogram	**FDA**	Food and Drug Administration
mg	milligram	**GLP**	Good Laboratory Practices
µg	microgram	**GMP**	Good Manufacturing Practices
ng	nanogram	**IACUC**	Institutional Animal Care and Use Committee
pg	picogram	**ICH**	International Conference on Harmonization
cm	centimeter	**MHLW**	Ministry of Health, Labor and Welfare
mm	millimeter	**NIEHS**	National Institute of Environmental Health Sciences
µm	micrometer	**NTP**	National Toxicology Program
sec	second	**OECD**	Organisation for Economic Co-operation and Development
min	minute	**PHS**	Public Health Service
h	hour	**QA**	Quality Assurance
d	day	**QAU**	Quality Assurance Unit
wk	week	**SOP**	Standard Operating Procedures
rpm	revolutions per minute	**USDA**	United States Department of Agriculture
NBF	neutral buffered formalin	**LCA**	Laboratory Corporation of America
No.	number	**PAI**	Pathology Associates, A Charles River Company
NA	not applicable	**RACB**	reproductive assessment by continuous breeding
N	number		

Table 1
Summary of Animal Disposition and Clinical Observations
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Day Numbers Relative to Start Date

	Group 1	Group 2
Sex: Male		
Animal Disposition		
Terminal Kill		
Number of Observations	3	5
Number of Animals	3	5
Days from - to	11 11	11 11
Recovery Kill 1		
Number of Observations	3	5
Number of Animals	3	5
Days from - to	61 61	61 61
Recovery Kill 2		
Number of Observations	3	5
Number of Animals	3	5
Days from - to	91 91	91 91
Clinical Observations		
Urine stain		
Number of Observations	.	1
Number of Animals	.	1
Days from - to	.	91 91

. – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Table 1 (continued)
Summary of Animal Disposition and Clinical Observations
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Day Numbers Relative to Start Date

	Group 1	Group 2
Sex: Female		
Animal Disposition		
Terminal Kill		
Number of Observations	3	5
Number of Animals	3	5
Days from - to	11 11	11 11
Recovery Kill 1		
Number of Observations	3	5
Number of Animals	3	5
Days from - to	61 61	61 61
Recovery Kill 2		
Number of Observations	3	5
Number of Animals	3	5
Days from - to	91 91	91 91

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Table 2
Summary of Cageside Observations
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Cageside observations were performed twice daily. No abnormal cageside observations were noted.

Table 3
Summary of Body Weights (g)
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Group Sex		Day Numbers Relative to Start Date 1	8	11	15	22	29	36	43
1m	Mean	2815.8	3028.9	3141.3	3120.2	3204.5	3329.3	3402.2	3465.3
	S.D.	73.2	80.4	112.5	75.4	115.2	135.2	131.8	146.6
	N	9	9	3	6	6	6	6	6
2m	Mean	2831.8	3033.5	3202.8	3153.4	3303.5	3426.5	3525.6	3608.4
	S.D.	112.2	111.2	179.9	113.0	168.3	193.2	204.2	236.6
	N	15	15	5	10	10	10	10	10
1f	Mean	2699.6	2931.0	3128.3	3044.8	3133.2	3261.7	3340.3	3442.8
	S.D.	79.2	110.0	121.1	160.5	221.7	275.4	328.5	352.8
	N	9	9	3	6	6	6	6	6
2f	Mean	2747.6	2960.3	3018.8	3175.3	3372.1*	3507.7	3569.6	3652.5
	S.D.	123.5	133.9	247.2	111.9	150.9	194.3	215.6	225.6
	N	15	15	5	10	10	10	10	10

m – Male f – Female * – Significantly different from the control value, $p<0.05$

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Table 3 (continued)
Summary of Body Weights (g)
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Group Sex		Day Numbers Relative to Start Date 50	57	61	64	71	78	85	91
1m	Mean	3542.0	3609.7	3705.7	3612.3	3702.7	3776.0	3815.0	3847.0
	S.D.	183.0	206.8	293.5	149.8	209.8	181.1	216.5	157.6
	N	6	6	3	3	3	3	3	3
2m	Mean	3697.7	3745.5	3712.6	3851.2	3892.4	3941.2	4046.2	4096.2
	S.D.	260.6	251.7	308.2	196.1	204.6	229.0	239.2	249.3
	N	10	10	5	5	5	5	5	5
1f	Mean	3552.8	3616.2	3664.3	3645.0	3680.3	3743.7	3804.0	3806.0
	S.D.	366.9	406.5	634.3	353.5	412.2	434.0	470.7	493.9
	N	6	6	3	3	3	3	3	3
2f	Mean	3754.8	3853.3	4105.6	3792.2	3851.6	3910.0	3983.6	3802.2
	S.D.	259.7	234.3	225.4	198.0	224.7	217.6	236.8	136.5
	N	10	10	5	5	5	5	5	5

m – Male f - Female

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5 x 10^{11} virus particles

Table 4
Summary of Body Weight Changes (g)
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Day Numbers Relative to Start Date

Group Sex	Base Weight Day 1	From: To:	1 8	8 11	Absolute Change 1 11	Percent Change 1 11	8 15	15 22	22 29	29 36	36 43	43 50	50 57
1m	2815.8	Mean	213.1	90.0	319.3	11.3	102.5	84.3	124.8	72.8	63.2	76.7	67.7
	73.16	S.D.	23.75	14.73	36.83	1.15	18.40	70.03	22.91	27.32	30.32	64.95	48.69
	9	N	9	3	3	3	6	6	6	6	6	6	6
2m	2831.8	Mean	201.7	116.2	299.0	10.3	146.5	150.1	123.0	99.1	82.8	89.3	47.8
	112.18	S.D.	63.71	47.11	65.68	1.98	61.91	68.37	38.32	41.69	47.03	42.10	63.58
	15	N	15	5	5	5	10	10	10	10	10	10	10
1f	2699.6	Mean	231.4	147.7	368.3	13.3	138.7	88.3	128.5	78.7	102.5	110.0	63.3
	79.22	S.D.	58.58	76.25	115.52	4.15	68.76	73.54	58.02	57.40	33.60	26.24	40.18
	9	N	9	3	3	3	6	6	6	6	6	6	6
2f	2747.6	Mean	212.7	146.4	323.8	11.9	171.0	196.8*	135.6	61.9	82.9	102.3	98.5
	123.48	S.D.	56.37	97.70	131.47	4.59	84.33	57.13	54.05	74.11	45.69	53.88	33.39
	15	N	15	5	5	5	10	10	10	10	10	10	10

m – Male f – Female * – Significantly different from the control value, $p < 0.05$

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Table 4 (continued)
Summary of Body Weight Changes (g)
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Day Numbers Relative to Start Date

Group Sex	Base Weight Day 1	From: To:	57 61	Absolute Change 1 61	Percent Change 1 61	57 64	64 71	71 78	78 85	85 91	Absolute Change 1 91	Percent Change 1 91
1m	2815.8	Mean	54.7	909.3	32.5	44.0	90.3	73.3	39.0	32.0	1018.0	36.0
	73.16	S.D.	14.84	286.85	10.17	35.34	62.32	56.86	46.29	76.62	89.77	2.88
	9	N	3	3	3	3	3	3	3	3	3	3
2m	2831.8	Mean	9.0	892.6	31.5	63.8	41.2	48.8	105.0*	50.0	1324.6	47.8
	112.18	S.D.	38.37	230.01	7.34	29.66	23.34	55.30	16.54	24.73	248.09	9.33
	15	N	5	5	5	5	5	5	5	5	5	5
1f	2699.6	Mean	41.7	1011.0	37.7	35.3	35.3	63.3	60.3	2.0	1120.7	41.5
	79.22	S.D.	100.92	546.41	19.09	22.19	60.93	22.90	44.86	104.89	428.37	14.84
	9	N	3	3	3	3	3	3	3	3	3	3
2f	2747.6	Mean	108.8	1283.2	45.6	82.4	59.4	58.4	73.6	-181.4	1076.8	39.5
	123.48	S.D.	25.17	212.44	7.90	53.10	53.10	45.74	37.24	112.81	125.97	4.80
	15	N	5	5	5	5	5	5	5	5	5	5

m – Male f – Female * – Significantly different from the control value, $p < 0.05$

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Appendix 1
Certificates of Analysis
Ad26 (b) (4) 91-Day Intramuscular Single Dose
Biodistribution Study in New Zealand White Rabbits

Crucell

Certificate of Analysis

Ad26 (b) (4) Placebo
(b) (4)

[redacted]

Crucell

Certificate of Analysis

Ad26 (b) (4) **Placebo**

(b) (4), (b) (6)

(b) (4)

Cobra Biologics

Certificate of Analysis

Ad26 (b) (4) **Drug Product for toxicity studies**

(b) (4)

Crucell

Certificate of Analysis

Ad26 (b) (4) **Drug Product for toxicity studies**

(b) (4), (b) (6)

(b) (4)

Appendix 2
Stability Reports
Ad26 (b) (4) 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Crucell

Crucell

DATA REPORT (*in vitro* study)

TITLE: **Stability study of Ad26 (b) (4) Drug Product stored at room temperature**

(b) (4), (b) (6)

Crucell

(b) (4)

Crucell

(b) (4), (b) (6)

Crucell

(b) (4)

Crucell

(b) (4)

Crucell

(b) (4)

Crucell

(b) (4)

Crucell

(b) (4)

Crucell

(b) (4)

Crucell

(b) (4)

Crucell

Attachment 3: SPSS syntax

Syntax used for SPSS statistical analysis:

```
COMPUTE start_dat = LAG(data) .
EXECUTE .

COMPUTE dif = data-start_dat .
EXECUTE .

USE ALL.
COMPUTE filter_$=(tijd = 4).
VARIABLE LABEL filter_$ 'tijd = 4 (FILTER)'.
VALUE LABELS filter_$  0 'Not Selected' 1 'Selected'.
FORMAT filter_$ (f1.0).
FILTER BY filter_$.
EXECUTE .

T-TEST
 /TESTVAL = 0
 /MISSING = ANALYSIS
 /VARIABLES = dif
 /CRITERIA = CI(.95) .
```

Crucell

(b) (4), (b) (6)

Crucell

(b) (4)

Crucell

(b) (4)

Crucell

(b) (4)

2 Scientific Review

Responsible Scientist:

Name: (b) (6) Sign: (b) (6) Date: 12 June 07

Peer Reviewer:

Name: (b) (6) Sign: (b) (6) Date: 12 Jun 07

Qualified Person:

Name: (b) (6) Sign: (b) (6) Date: 12 June 07

Crucell

(b) (4)

Crucell

(b) (4)

Crucell

(b) (4)

Crucell

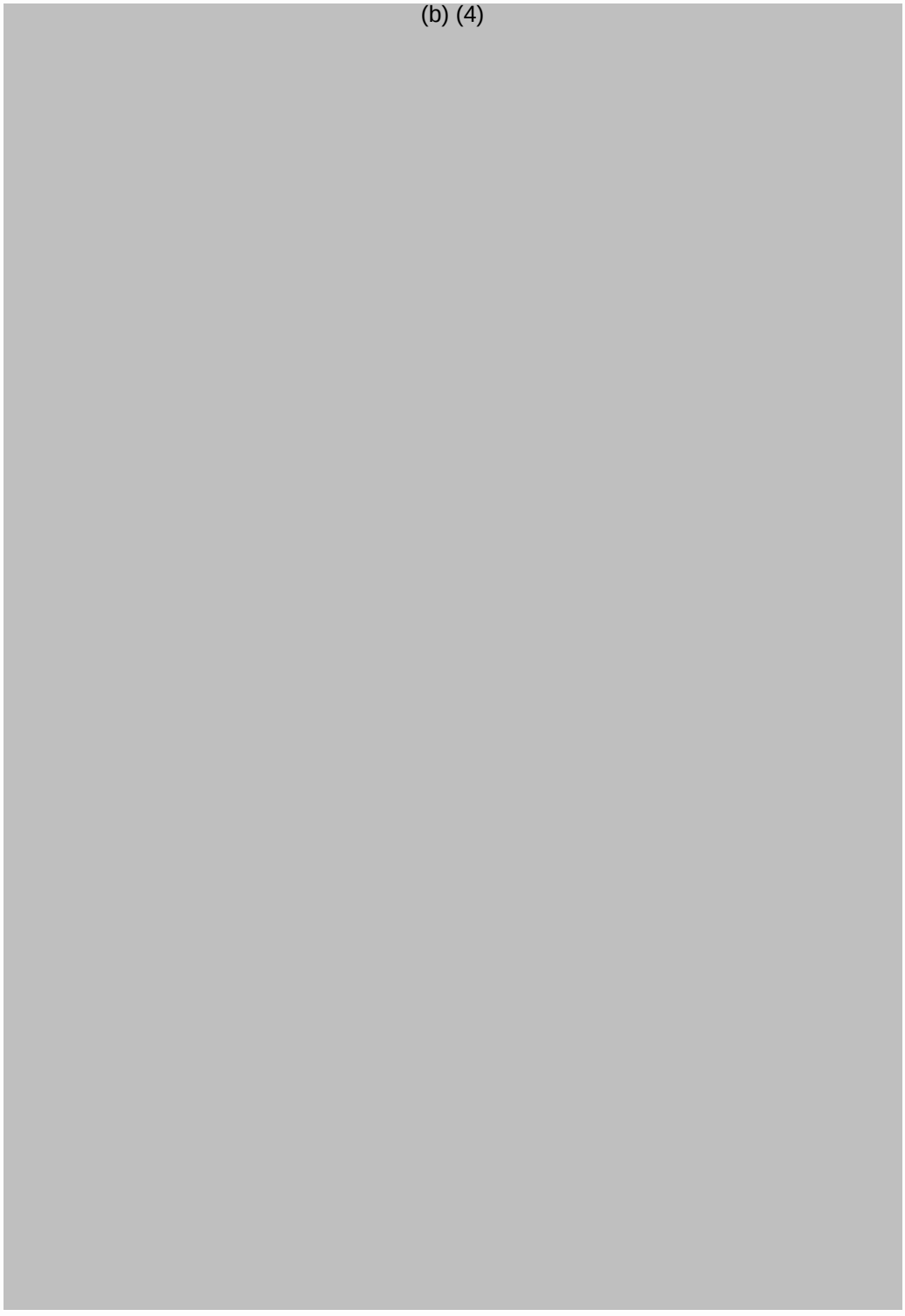
(b) (4)

Crucell

(b) (4)

Crucell

(b) (4)



Crucell

(b) (4)

Crucell

(b) (4)

7 Attachments

7.1. Certificate of Analysis (b) (4)

Appendix 3
Individual Animal Disposition and Clinical Observations
Ad26 (b) (4) 91-Day Intramuscular Single Dose
Biodistribution Study in New Zealand White Rabbits

Appendix 3
Individual Animal Disposition and Clinical Observations
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Day Numbers Relative to Start Date

Group Sex	Animal Number	Clinical Sign	1	8	11	15	22	29	36	43	50	57	61	64	71	78	85	91
1m	14530	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14531	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14532	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14533	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14534	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14535	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14536	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14537	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14538	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

m – Male X – Present . – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Appendix 3 (continued)
Individual Animal Disposition and Clinical Observations
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Group Sex	Animal Number	Clinical Sign	Day Numbers Relative to Start Date: 1	8	11	15	22	29	36	43	50	57	61	64	71	78	85	91
2m	14548	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14549	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14550	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14551	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14552	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14553	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14554	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14555	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14556	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14557	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14558	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14559	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	.
		Urine stain	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14560	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14561	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14562	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

m – Male X – Present . – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Appendix 3 (continued)
Individual Animal Disposition and Clinical Observations
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Group Sex	Animal Number	Clinical Sign	Day Numbers Relative to Start Date: 1	8	11	15	22	29	36	43	50	57	61	64	71	78	85	91
1f	14539	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14540	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14541	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14542	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14543	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14544	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14545	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14546	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14547	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

f – Female X – Present . – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Appendix 3 (continued)
Individual Animal Disposition and Clinical Observations
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Day Numbers Relative to Start Date

Group Sex	Animal Number	Clinical Sign	1	8	11	15	22	29	36	43	50	57	61	64	71	78	85	91
2f	14563	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14564	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14565	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14566	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14567	No Abnormalities Detected	X	X	X	.	.	.	.	.	.	.	.	.	.	.	.	.
		Terminal Kill	.	.	X	.	.	.	.	.	.	.	.	.	.	.	.	.
	14568	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14569	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14570	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14571	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14572	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	X	.	.	.	.	.
		Recovery Kill 1	.	.	.	.	.	.	.	.	.	.	X	.	.	.	.	.
	14573	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14574	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14575	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14576	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X
	14577	No Abnormalities Detected	X	X	.	X	X	X	X	X	X	X	.	X	X	X	X	X
		Recovery Kill 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	X

f – Female X – Present . – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Appendix 4
Individual Body Weights
Ad26 (b) (4) 91-Day Intramuscular Single Dose
Biodistribution Study in New Zealand White Rabbits

Appendix 4
Individual Body Weights (g)
Ad26 (b) (4): 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Group Sex	Animal Number	Day Numbers Relative to Start Date 1	8	11	15	22	29	36	43	50	57	61	64	71	78	85	91
1m	14530	2803	3010	3083	.	.	.	.	.	.	.	.	.	.	.	.	.
	14531	2745	2971	3070	.	.	.	.	.	.	.	.	.	.	.	.	.
	14532	2918	3173	3271	.	.	.	.	.	.	.	.	.	.	.	.	.
	14533	2734	2943	.	3062	3170	3305	3415	3495	3618	3653	3704	.	.	.	.	.
	14534	2839	3070	.	3181	3371	3512	3557	3619	3798	3929	4000	.	.	.	.	.
	14535	2816	2985	.	3054	3033	3117	3198	3246	3301	3371	3413	.	.	.	.	.
	14536	2855	3059	.	3175	3243	3370	3438	3550	3546	3668	.	3739	3895	3905	3938	3975
	14537	2720	2937	.	3041	3148	3263	3303	3326	3365	3390	.	3447	3479	3569	3565	3671
	14538	2912	3112	.	3208	3262	3409	3502	3556	3624	3647	.	3651	3734	3854	3942	3895
2m	14548	2890	3158	3226	.	.	.	.	.	.	.	.	.	.	.	.	.
	14549	2688	2864	2936	.	.	.	.	.	.	.	.	.	.	.	.	.
	14550	3050	3293	3443	.	.	.	.	.	.	.	.	.	.	.	.	.
	14551	2964	3023	3198	.	.	.	.	.	.	.	.	.	.	.	.	.
	14552	2927	3095	3211	.	.	.	.	.	.	.	.	.	.	.	.	.
	14553	2704	2908	.	2981	3032	3164	3264	3322	3376	3453	3516	.	.	.	.	.
	14554	2948	3107	.	3241	3353	3497	3584	3687	3839	3944	3978	.	.	.	.	.
	14555	2749	2935	.	3049	3129	3206	3291	3339	3371	3469	3450	.	.	.	.	.
	14556	2887	3080	.	3310	3566	3739	3866	4010	4152	4117	4112	.	.	.	.	.
	14557	2812	2953	.	3059	3167	3251	3327	3422	3490	3535	3507	.	.	.	.	.
	14558	2797	3085	.	3179	3354	3422	3499	3565	3634	3544	.	3653	3677	3768	3876	3918
	14559	2718	3024	.	3125	3236	3358	3421	3430	3529	3582	.	3633	3667	3632	3713	3750
	14560	2696	2957	.	3169	3412	3531	3717	3769	3880	3939	.	3967	4046	4150	4258	4331
	14561	2773	2945	.	3099	3279	3408	3551	3637	3760	3868	.	3937	3984	4038	4165	4243
	14562	2874	3075	.	3322	3507	3689	3736	3903	3946	4004	.	4066	4088	4118	4219	4239

m – Male . – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5 x 10^{11} virus particles

Appendix 4 (continued)
Individual Body Weights (g)
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Group Sex	Animal Number	Day Numbers Relative to Start Date 1	8	11	15	22	29	36	43	50	57	61	64	71	78	85	91
1f	14539	2771	3039	3256	.	.	.	.	.	.	.	.	.	.	.	.	.
	14540	2761	2949	3015	.	.	.	.	.	.	.	.	.	.	.	.	.
	14541	2748	2954	3114	.	.	.	.	.	.	.	.	.	.	.	.	.
	14542	2766	3105	.	3256	3471	3671	3822	3970	4118	4242	4359	.	.	.	.	.
	14543	2587	2793	.	2943	3030	3112	3203	3267	3390	3437	3518	.	.	.	.	.
	14544	2607	2752	.	2856	2911	2974	2992	3058	3173	3189	3116	.	.	.	.	.
	14545	2626	2898	.	3047	3065	3225	3299	3429	3534	3608	.	3640	3695	3751	3843	3726
	14546	2654	2917	.	2951	2980	3067	3076	3180	3249	3279	.	3294	3261	3306	3315	3357
	14547	2776	2972	.	3216	3342	3521	3650	3753	3853	3942	.	4001	4085	4174	4254	4335
2f	14563	2494	2629	2717	.	.	.	.	.	.	.	.	.	.	.	.	.
	14564	2704	2842	2858	.	.	.	.	.	.	.	.	.	.	.	.	.
	14565	2606	2841	3037	.	.	.	.	.	.	.	.	.	.	.	.	.
	14566	2768	2959	3122	.	.	.	.	.	.	.	.	.	.	.	.	.
	14567	2903	3091	3360	.	.	.	.	.	.	.	.	.	.	.	.	.
	14568	2979	3179	.	3409	3667	3851	4007	4111	4259	4327	4438	.	.	.	.	.
	14569	2708	2907	.	3187	3353	3459	3608	3672	3759	3860	3954	.	.	.	.	.
	14570	2924	3136	.	3218	3383	3533	3527	3583	3712	3776	3857	.	.	.	.	.
	14571	2764	2989	.	3253	3468	3666	3588	3793	4006	4045	4193	.	.	.	.	.
	14572	2737	2925	.	3209	3499	3701	3753	3808	3881	3976	4086	.	.	.	.	.
	14573	2725	2938	.	3032	3128	3231	3283	3345	3402	3531	.	3526	3533	3621	3644	3639
	14574	2660	3037	.	3126	3376	3477	3560	3623	3759	3865	.	3978	3985	4108	4155	3898
	14575	2802	2994	.	3140	3317	3441	3573	3637	3727	3844	.	3916	4037	4049	4162	3951
	14576	2679	2920	.	3019	3208	3236	3242	3333	3384	3535	.	3638	3696	3734	3824	3680
	14577	2761	3018	.	3160	3322	3482	3555	3620	3659	3774	.	3903	4007	4038	4133	3843

f – Female . – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Appendix 5
Individual Body Weight Changes
Ad26 (b) (4) 91-Day Intramuscular Single Dose
Biodistribution Study in New Zealand White Rabbits

Appendix 5
Individual Body Weight Changes (g)
Ad26 (b) (4): 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

				Day Numbers Relative to Start Date										
		Base Weight				Absolute Change	Percent Change							
Group	Animal	Day	From:	1	8	1	1	8	15	22	29	36	43	50
Sex	Number	1	To:	8	11	11	11	15	22	29	36	43	50	57
1m	14530	2803		207	73	280	9.989	.	.	.	.	.	.	.
	14531	2745		226	99	325	11.840	.	.	.	.	.	.	.
	14532	2918		255	98	353	12.097	.	.	.	.	.	.	.
	14533	2734		209	.	.	.	119	108	135	110	80	123	35
	14534	2839		231	.	.	.	111	190	141	45	62	179	131
	14535	2816		169	.	.	.	69	-21	84	81	48	55	70
	14536	2855		204	.	.	.	116	68	127	68	112	-4	122
	14537	2720		217	.	.	.	104	107	115	40	23	39	25
	14538	2912		200	.	.	.	96	54	147	93	54	68	23
2m	14548	2890		268	68	336	11.626	.	.	.	.	.	.	.
	14549	2688		176	72	248	9.226	.	.	.	.	.	.	.
	14550	3050		243	150	393	12.885	.	.	.	.	.	.	.
	14551	2964		59	175	234	7.895	.	.	.	.	.	.	.
	14552	2927		168	116	284	9.703	.	.	.	.	.	.	.
	14553	2704		204	.	.	.	73	51	132	100	58	54	77
	14554	2948		159	.	.	.	134	112	144	87	103	152	105
	14555	2749		186	.	.	.	114	80	77	85	48	32	98
	14556	2887		193	.	.	.	230	256	173	127	144	142	-35
	14557	2812		141	.	.	.	106	108	84	76	95	68	45
	14558	2797		288	.	.	.	94	175	68	77	66	69	-90
	14559	2718		306	.	.	.	101	111	122	63	9	99	53
	14560	2696		261	.	.	.	212	243	119	186	52	111	59
	14561	2773		172	.	.	.	154	180	129	143	86	123	108
	14562	2874		201	.	.	.	247	185	182	47	167	43	58

m – Male . – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Appendix 5 (continued)
Individual Body Weight Changes (g)
Ad26 (b) (4): 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Group Sex	Animal Number	Base Weight Day 1	Day Numbers Relative to Start Date From:	57	Absolute Change 1	Percent Change 1	57	64	71	78	85	Absolute Change 1	Percent Change 1
			To:	61	61	61	64	71	78	85	91	91	91
1m	14530	2803		.	.	.	.	.	.	.	.	.	.
	14531	2745		.	.	.	.	.	.	.	.	.	.
	14532	2918		.	.	.	.	.	.	.	.	.	.
	14533	2734		51	970	35.479	.	.	.	.	.	.	.
	14534	2839		71	1161	40.895	.	.	.	.	.	.	.
	14535	2816		42	597	21.200	.	.	.	.	.	.	.
	14536	2855		.	.	.	71	156	10	33	37	1120	39.229
	14537	2720		.	.	.	57	32	90	-4	106	951	34.963
	14538	2912		.	.	.	4	83	120	88	-47	983	33.757
2m	14548	2890		.	.	.	.	.	.	.	.	.	.
	14549	2688		.	.	.	.	.	.	.	.	.	.
	14550	3050		.	.	.	.	.	.	.	.	.	.
	14551	2964		.	.	.	.	.	.	.	.	.	.
	14552	2927		.	.	.	.	.	.	.	.	.	.
	14553	2704		63	812	30.030	.	.	.	.	.	.	.
	14554	2948		34	1030	34.939	.	.	.	.	.	.	.
	14555	2749		-19	701	25.500	.	.	.	.	.	.	.
	14556	2887		-5	1225	42.432	.	.	.	.	.	.	.
	14557	2812		-28	695	24.716	.	.	.	.	.	.	.
	14558	2797		.	.	.	109	24	91	108	42	1121	40.079
	14559	2718		.	.	.	51	34	-35	81	37	1032	37.969
	14560	2696		.	.	.	28	79	104	108	73	1635	60.645
	14561	2773		.	.	.	69	47	54	127	78	1470	53.011
	14562	2874		.	.	.	62	22	30	101	20	1365	47.495

m – Male . – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Appendix 5 (continued)
Individual Body Weight Changes (g)
Ad26 (b) (4): 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

			Day Numbers Relative to Start Date										
		Base				Absolute	Percent							
		Weight				Change	Change							
Group	Animal	Day	From:	1	8	1	1	8	15	22	29	36	43	50
Sex	Number	1	To:	8	11	11	11	15	22	29	36	43	50	57
1f	14539	2771		268	217	485	17.503	.	.	.	.	.	.	.
	14540	2761		188	66	254	9.200	.	.	.	.	.	.	.
	14541	2748		206	160	366	13.319	.	.	.	.	.	.	.
	14542	2766		339	.	.	.	151	215	200	151	148	148	124
	14543	2587		206	.	.	.	150	87	82	91	64	123	47
	14544	2607		145	.	.	.	104	55	63	18	66	115	16
	14545	2626		272	.	.	.	149	18	160	74	130	105	74
	14546	2654		263	.	.	.	34	29	87	9	104	69	30
	14547	2776		196	.	.	.	244	126	179	129	103	100	89
2f	14563	2494		135	88	223	8.941	.	.	.	.	.	.	.
	14564	2704		138	16	154	5.695	.	.	.	.	.	.	.
	14565	2606		235	196	431	16.539	.	.	.	.	.	.	.
	14566	2768		191	163	354	12.789	.	.	.	.	.	.	.
	14567	2903		188	269	457	15.742	.	.	.	.	.	.	.
	14568	2979		200	.	.	.	230	258	184	156	104	148	68
	14569	2708		199	.	.	.	280	166	106	149	64	87	101
	14570	2924		212	.	.	.	82	165	150	-6	56	129	64
	14571	2764		225	.	.	.	264	215	198	-78	205	213	39
	14572	2737		188	.	.	.	284	290	202	52	55	73	95
	14573	2725		213	.	.	.	94	96	103	52	62	57	129
	14574	2660		377	.	.	.	89	250	101	83	63	136	106
	14575	2802		192	.	.	.	146	177	124	132	64	90	117
	14576	2679		241	.	.	.	99	189	28	6	91	51	151
	14577	2761		257	.	.	.	142	162	160	73	65	39	115

f – Female . – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Appendix 5 (continued)
Individual Body Weight Changes (g)
Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

Group Sex	Animal Number	Base Weight Day 1	Day Numbers Relative to Start Date From:	57	Absolute Change 1	Percent Change 1	57	64	71	78	85	Absolute Change 1	Percent Change 1
			To:	61	61	61	64	71	78	85	91	91	91
1f	14539	2771		.	.	.	.	.	.	.	.	.	.
	14540	2761		.	.	.	.	.	.	.	.	.	.
	14541	2748		.	.	.	.	.	.	.	.	.	.
	14542	2766		117	1593	57.592	.	.	.	.	.	.	.
	14543	2587		81	931	35.988	.	.	.	.	.	.	.
	14544	2607		-73	509	19.524	.	.	.	.	.	.	.
	14545	2626		.	.	.	32	55	56	92	-117	1100	41.889
	14546	2654		.	.	.	15	-33	45	9	42	703	26.488
	14547	2776		.	.	.	59	84	89	80	81	1559	56.160
2f	14563	2494		.	.	.	.	.	.	.	.	.	.
	14564	2704		.	.	.	.	.	.	.	.	.	.
	14565	2606		.	.	.	.	.	.	.	.	.	.
	14566	2768		.	.	.	.	.	.	.	.	.	.
	14567	2903		.	.	.	.	.	.	.	.	.	.
	14568	2979		111	1459	48.976	.	.	.	.	.	.	.
	14569	2708		94	1246	46.012	.	.	.	.	.	.	.
	14570	2924		81	933	31.908	.	.	.	.	.	.	.
	14571	2764		148	1429	51.700	.	.	.	.	.	.	.
	14572	2737		110	1349	49.288	.	.	.	.	.	.	.
	14573	2725		.	.	.	-5	7	88	23	-5	914	33.541
	14574	2660		.	.	.	113	7	123	47	-257	1238	46.541
	14575	2802		.	.	.	72	121	12	113	-211	1149	41.006
	14576	2679		.	.	.	103	58	38	90	-144	1001	37.365
	14577	2761		.	.	.	129	104	31	95	-290	1082	39.189

f – Female . – Not applicable

Nominal Dose: Group 1 – 0 virus particles
Group 2 – 0.5×10^{11} virus particles

Appendix 6
Biodistribution Report
Ad26 (b) (4) 91-Day Intramuscular Single Dose
Biodistribution Study in New Zealand White Rabbits

(b) (4)

(b) (4)

REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION ANALYSIS OF THE BIODISTRIBUTION OF Ad26 (b) (4) IN RABBIT

ABSTRACT: A qualified quantitative polymerase chain reaction (qPCR) assay was used to detect and quantify Ad26 (b) (4) in tissues and blood collected from (b) (4) (b) (4) ; a biodistribution study in rabbit. The assay detects a 162 base pair sequence, unique to the vector, using the ABI Prism 7700 Sequence Detection System. The number of copies of vector detected in up to one microgram of genomic DNA extracted from each tissue was quantified using serial dilutions of plasmid DNA containing the target sequence as standards. The lower limit of detection of the assay is 10 copies of Ad26 (b) (4) µg DNA; the lower limit of quantification is 50 copies of Ad26 (b) (4) µg DNA.

Prepared For: Beth Israel Deaconess Medical Center

(b) (4)

Operator:	(b) (4), (b) (6)	(b) (4), (b) (6)	Date: 06.27.07
Operator:			Date: 06.27.07
Operator:			Date: 06.27.07
Operator:			Date: 06.27.07
Operator:			Date: 06.27.07
Operator:			Date: 06.27.07
Principal Investigator:	(b) (4), (b) (6)		Date: 06.27.07
Quality Assurance:	(b) (4), (b) (6)		Date: 06.27.07

(b) (4)

Table of Contents

I. Study Information

(b) (4) **Statement of Work:** (b) (4)

Specimen Identification: Rabbit tissues and blood collected from (b) (4) Study (b) (4)

Objective: The objective of the study was to measure the number of copies of Ad26 (b) (4) per microgram of DNA purified from rabbit tissues and blood.

Sponsor: Beth Israel Deaconess Medical Center
Division of Viral Pathogenesis
41 Avenue Louis Pasteur
Boston, MA 02115

Sponsor Representative: (b) (4), (b) (6)
(b) (4), (b) (6)

Test Facility: (b) (4)

Study Director: (b) (4), (b) (6)
(b) (4), (b) (6)

Test Site: (b) (4)

Principal Investigator: (b) (4), (b) (6)
(b) (4), (b) (6)

Study Phase Schedule

Study Phase Initiation: 05/09/2007
Analysis Initiation: 05/10/2007
Analysis Completion: 06/04/2007
Study Phase Completion: The date of the Principal Investigator's signature in the *Principal Investigator's Approval* section of this study phase report.

Archives: Raw data, records, the statement of work, and a copy of the report will be maintained by (b) (4) Quality Assurance Department as described in (b) (4) Standard Operating Procedure (SOP) No. (b) (4) (b) (4)

(b) (4)

II. Assay Description and Methods

A. Assay Description

A TaqMan based assay was qualified to detect and quantify Ad26 (b) (4) DNA sequence in rabbit tissue. The assay design amplifies (b) (4) of the Ad26 (b) (4) DNA sequence. It was optimized to provide maximum sensitivity and specificity for detecting the target gene sequence in a background of rabbit tissue genomic DNA (gDNA). (b) (4)

B. Preparation of Standards

DNA standards were prepared by serially diluting Sponsor provided pAdapt26 (b) (4) plasmid DNA (b) (4) in a background of gDNA isolated from rabbit liver and prepared such that 1 µg of background gDNA was present per PCR. A dilution series was prepared to span the quantitative range of the assay with the following points: $1x10^6$ copies of vector DNA per microgram of animal model genomic DNA (copies/µg DNA), $1x10^5$ copies/µg DNA, $1x10^4$ copies/µg DNA, $1x10^3$ copies/µg DNA, $1x10^2$ copies/µg DNA and 50 copies/µg DNA. In addition to the standard curve, a point representing the assay's limit of detection of the assay is 10 copies of Ad26 (b) (4) /µg DNA.

C. Preparation of Specimens

DNA was extracted from tissue and blood specimens as described in (b) (4) (b) (4) *Operation and Maintenance of the BioRobot M48 Nucleic Acid Extraction System,* and/or (b) (4) *Isolation of Genomic DNA From Tissues or Biological Fluids*. A naive tissue, provided by (b) (4) was included with each batch of specimens to serve as an extraction contamination control (NEC-negative extraction control). The concentration of the DNA purified from each tissue was determined by absorbance at 260 nm (A260) according to (b) (4) (b) (4) *Determination of Nucleic Acid Concentration by Spectrophotometry* and the concentration subsequently adjusted to 100 ng/µL with water. Ten microliters (1 µg DNA) were used in each qPCR. For samples with DNA concentrations less than 100 ng/µL but greater than or equal to 50 ng/µL, the DNA was adjusted to 50 ng/µL, and

(b) (4)

20 µL (1 µg DNA) of the DNA preparation was run per reaction. Samples with DNA concentrations less than 50 ng/µL were run using 20 µL per qPCR. The mass or volume of DNA analyzed per PCR was recorded for each specimen and is reported in Appendix 1 of this report. Blood samples were run volumetrically with DNA from the equivalent of 10 microliters of blood analyzed in each reaction.

D. Real-Time Quantitative Polymerase Chain Reaction

qPCR amplification and fluorescence detection was performed using the ABI PRISM 7700 Sequence Detection System as described in (b) (4) *Operation of the ABI Sequence Detection System and Sequence Detector Software*, and (b) (4) *Quantitation of Target Sequences Using Universal Master Mix*. Three replicate qPCR reactions were performed on each specimen's DNA using the oligonucleotide primers and fluorescent probe described in the assay development report (b) (4) One of the three replicate reactions was spiked with 100 copies of vector to check for the presence of qPCR inhibitors. In addition to specimen DNA, each qPCR plate was run with one set of standards, a naïve rabbit genomic DNA negative control (0 copy) and the qPCR reagent control (NTC). Each extraction control (NEC) was included on at least one run with its corresponding specimens. These controls monitor the potential for non-specific amplification of animal model genomic DNA, contamination of the qPCR reagents, and contamination of specimen DNA during the extraction process, respectively. All controls were run in duplicate reactions.

E. Calculations and Data Analysis

For each qPCR run, the Sequence Detector Software v1.6.3 created a standard curve by plotting the mean C_T value (the cycle at which the reporter signal can be detected above baseline fluorescence) of each standard versus (LogN) starting copy number assigned to each standard. The software then performed a linear regression analysis to calculate the number of copies of the target sequence detected in each reaction for each specimen. The mean copy number of the duplicate reactions for each specimen was then calculated and the individual well and mean copy numbers were reported.

(b) (4)

5

Data generated by the Sequence Detection Software was copied into a Microsoft Excel worksheet. Data for reactions containing less than one microgram of gDNA were mathematically adjusted to final reporting units of the number of copies per one microgram DNA. The mass or volume of DNA analyzed per reaction can be found in Appendix 1 of this report.

F. Acceptance Criteria

Acceptability of a qPCR assay was determined by the following criteria:

The correlation coefficient of the standard curve must be $\geq$0.980.

The Negative Extraction Control (NEC) must test below the limit of detection of the assay.

The qPCR reagent control (NTC) must test below the limit of detection of the assay by at least 10-fold.

Acceptability of the result for an individual specimen under analysis was determined by the following criteria:

For specimens with a quantifiable number of copies of the target sequence (within the range of quantification) the difference between C_T values of the duplicate reactions used for quantification must be less than or equal to 1 C_T.

The C_T of the spiked reaction must be less than the mean C_T of the limit of detection.

(b) (4)

III. Results

The number of copies of Ad26 (b) (4) per microgram of genomic DNA purified from each tissue is reported in Tables 1 and 2. The quantification of Ad26 (b) (4) in blood is expressed per 10 µL of blood. Specimens testing below the limit of detection of the assay are identified as "LLD" (less than the limit of detection). Specimens testing greater than 10 but less than 50 copies are detectable below the limit of quantification of the assay and are identified as "NQ" (not quantifiable). Inhibited reactions were re-analyzed using less DNA and results mathematically adjusted to copies per µg. Results of repeat analyses were multiplied by the appropriate dilution factor and reported. The mass of DNA analyzed in each reaction appears in Appendix 1.

(b) (4)

7

Table 1- Biodistribution of Ad26 (b) (4) DNA at SD 11 and 61

Study Day	Treatment	Sex	Animal No.	Blood	Gonads	Liver	Thymus	Heart	Lung	Kidney	Spleen	Lymph Nodes		Bone Marrow	Brain	Injection Sites	
												Mesenteric	Iliac			Skin	Muscle
11	Placebo	Male	14530	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14531	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14532	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
		Female	14539	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14540	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14541	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
	Ad26 (b) (4) 0.5×10^{11} vp	Male	14548	LLD	LLD	LLD	LLD	LLD	LLD	LLD	50	LLD	960	LLD	LLD	LLD	LLD
			14549	LLD	LLD	LLD	LLD	LLD	LLD	LLD	92	LLD	119	LLD	LLD	LLD	5380
			14550	LLD	LLD	LLD	LLD	LLD	LLD	LLD	NQ	LLD	2439	LLD	LLD	LLD	61
			14551	LLD	LLD	LLD	LLD	LLD	LLD	LLD	95	LLD	2337	LLD	LLD	LLD	LLD
			14552	LLD	LLD	LLD	LLD	LLD	LLD	LLD	NQ	LLD	290	LLD	LLD	LLD	163
		Female	14563	LLD	LLD	LLD	LLD	LLD	LLD	LLD	NQ	LLD	8676	LLD	LLD	LLD	11981
			14564	LLD	LLD	LLD	LLD	LLD	LLD	LLD	116	LLD	4013	LLD	LLD	LLD	433
			14565	LLD	LLD	LLD	LLD	LLD	LLD	LLD	NQ	LLD	753	LLD	LLD	NQ	3313
			14566	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	456	LLD	LLD	LLD	2202
			14567	LLD	LLD	LLD	LLD	LLD	LLD	LLD	NQ	LLD	345	LLD	LLD	LLD	NQ
61	Placebo	Male	14533	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14534	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14535	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
		Female	14542	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14543	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14544	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
	Ad26 (b) (4) 0.5×10^{11} vp	Male	14553	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	115	LLD	LLD	LLD	LLD
			14554	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14555	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	NQ	LLD	LLD	LLD	LLD
			14556	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14557	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	84	LLD	LLD	LLD	LLD
		Female	14568	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14569	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14570	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	1400	LLD	LLD	LLD	NQ
			14571	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	239	LLD	LLD	LLD	NQ
			14572	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD

LLD = Lower than Limit of Detection (< 10 copies);
NQ = Not Quantifiable (> 10 copies and < 50 copies)

(b) (4)

8

Table 2- Biodistribution of Ad26 (b) (4) DNA at SD 91

Study Day	Treatment	Sex	Animal No.	Blood	Gonads	Liver	Thymus	Heart	Lung	Kidney	Spleen	Lymph Nodes		Bone Marrow	Brain	Injection Sites	
												Mesenteric	Iliac			Skin	Muscle
91	Placebo	Male	14536	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14537	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14538	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
		Female	14545	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14546	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14547	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
	Ad26 (b) (4) 0.5×10^{11} vp	Male	14558	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14559	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14560	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	120
			14561	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	50	LLD	LLD	LLD	LLD
			14562	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	1807	LLD	LLD	LLD	LLD
		Female	14573	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	NQ
			14574	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14575	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14576	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD
			14577	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD	LLD

LLD = Lower than Limit of Detection (< 10 copies)
NQ = Not Quantifiable (> 10 copies and < 50 copies)

(b) (4)

IV. Conclusion

A TaqMan™ based qPCR assay was used to measure the biodistribution and persistence of Ad26 (b) (4) in a study conducted in rabbit (b) (4) Results of the qPCR analysis determined that the vaccine was primarily localized in the spleen, iliac lymph node, and the muscle at the site of injection. By study day 61, the vaccine was no longer detected in the spleen. By study day 91, detection of the vector in the iliac lymph nodes and muscle at the site of injection decreased to 2 out of 10 treated animals.

(b) (4)

V. Principal Investigator's Approval

This phase of (b) (4) was performed in compliance with Title 21 of the U.S. Code of Federal Regulations, Part 58, *Good Laboratory Practice for Nonclinical Laboratory Studies*, as applicable and according to (b) (4) (b) (4)

(b) (4), (b) (6)

Principal Investigator

06.27.07
Date

(b) (4)

11

VI. Quality Assurance Statement

(b) (4) has been inspected and audited by the Quality Assurance Department of (b) (4) and as far as can be reasonably established, the methods described and the results incorporated in the report accurately reflect the raw data produced during the study. There were no deviations reported during the conduct of this study.

This study was subject to Quality Assurance inspection(s) and/or audit(s) as follows:

Inspection/Audit	Inspection/Audit Date	Date Reported to Management
Critical Phase Audit	02/22/07	02/22/07
Critical Phase Audit	04/10/07	04/10/07
Critical Phase Audit	05/08/07	05/08/07
Critical Phase Audit	05/30/07	06/01/07
Draft Report Audit	06/13/07	06/14/07
Final Report Audit	06/27/07	06/27/07

I certify that this study report provides a true and complete record of the data generated.

(b) (4), (b) (6)
Quality Assurance

06.27.07
Date

(b) (4)

12

Appendix 1 – DNA Analyzed per Reaction

Study Day	Treatment	Sex	Animal No.	Blood	Gonads	Liver	Thymus	Heart	Lung	Kidney	Spleen	Lymph Nodes		Bone Marrow	Brain	Injection Sites	
												Mesenteric	Iliac			Skin	Muscle
11	Placebo	Male	14530	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.71	1.00	1.00
			14531	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14532	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00
		Female	14539	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00
			14540	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14541	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Ad26 (b) (4) 0.5×10^{11} vp	Male	14548	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14549	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14550	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14551	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14552	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		Female	14563	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.77	1.00	1.00
			14564	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14565	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14566	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14567	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
61	Placebo	Male	14533	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.53	1.00	0.90
			14534	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.45	1.00	0.36	1.00	1.00
			14535	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.44	1.00	1.00
		Female	14542	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.37	1.00	1.00
			14543	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.39	1.00	1.00
			14544	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Ad26 (b) (4) 0.5×10^{11} vp	Male	14553	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.63	1.00	1.00
			14554	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14555	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.92	1.00	1.00
			14556	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14557	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.49	1.00	1.00
		Female	14568	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98
			14569	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.65	1.00	1.00	1.00	0.92
			14570	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.28	1.00	1.00
			14571	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98
			14572	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Data expressed as micrograms (µg) for tissue and microliter (µL) for blood

(b) (4)

13

Appendix 1 – Continued

Study Day	Treatment	Sex	Animal No.	Blood	Gonads	Liver	Thymus	Heart	Lung	Kidney	Spleen	Lymph Nodes		Bone Marrow	Brain	Injection Sites	
												Mesenteric	Iliac			Skin	Muscle
91	Placebo	Male	14536	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.61	1.00	1.00
			14537	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.11	1.00	1.00	1.00	1.00
			14538	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.45	1.00	1.00	1.00	1.00
		Female	14545	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96	1.00	1.00	1.00	1.00
			14546	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50	1.00	1.00
			14547	10	1.00	1.00	0.36	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.51
	Ad26 (b) (4) 0.5×10^{11} vp	Male	14558	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14559	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14560	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14561	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.32	1.00	1.00
			14562	10	1.00	1.00	1.00	1.00	0.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
		Female	14573	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14574	10	1.00	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14575	10	1.00	1.00	1.00	1.00	0.91	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
			14576	10	1.00	1.00	1.00	0.97	1.00	1.00	1.00	0.69	0.57	1.00	0.49	1.00	1.00
			14577	10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00	1.00

Data expressed as micrograms (µg) for tissue and microliter (µL) for blood

(b) (4)

14

Appendix 7
Protocol, Amendments, and Deviations
Ad26 (b) (4) 91-Day Intramuscular Single Dose
Biodistribution Study in New Zealand White Rabbits

(b) (4)

STUDY PROTOCOL

Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

(b) (4)

APPROVALS

(b) (4)

(b) (4), (b) (6) 1/8/07 Date
Senior Study Director

Beth Israel Deaconess:

(b) (4), (b) (6) 1/7/07 Date
Sponsor's Representative

(b) (4), (b) (6) 1-15-07 Date
(b) (4), (b) (6)
Vice President, Toxicology

(b) (4)

Beth Israel Deaconess (b) (4)

PROTOCOL

I. Study Title

Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits

II. Purpose

The purpose of this study is to determine the biodistribution of an adenovector-based (b) (4) vaccine in New Zealand White rabbits during a 91-day study period when administered by a single intramuscular injection.

III. III. Test Article Summary

The test article is purified, replication-incompetent, recombinant Adenovirus serotype 26 that expresses the clade A (b) (4) protein.

IV. Sponsor Information

A. Name and Address

Beth Israel Deaconess Medical Center
Division of Viral Pathogenesis
Research East Room 213
41 Avenue Louis Pasteur
Boston, MA 02115

B. Sponsor's Representative

(b) (4), (b) (6)

V. (b) (4)

A. Study Director

(b) (4), (b) (6)

Beth Israel Deaconess (b) (4)

B. Alternate Study Contact: (b) (4)

VI. Test Sites, Designated Archive Facilities and Contributing Scientists

A. Toxicology (b) (4)

B. Chemical Archive Facility

Crucell Holland BV
PO Box 2048
2301 CA Leiden
The Netherlands

C. Designated Archive Facility (b) (4)

D. Biodistribution Analysis (b) (4)

VII. Regulatory Information

A. Compliance

This study will be conducted according to the protocol and the company's Standard Operating Procedures (SOP). Portions of the study performed by the Sponsor or subcontractor(s) will be performed according to the protocol and their SOPs.

This study will be conducted in compliance with current U.S. FDA Good Laboratory Practice (GLP) Regulations for Non-clinical Laboratory Studies (21 CFR Part 58).

B. Quality Assurance

(b) (4) Quality Assurance Unit (QAU) will audit the study in accordance with the SOPs and GLPs.

Beth Israel Deaconess (b) (4)

Any portions of the study performed by the Sponsor or subcontractor(s) will be verified by their QAUs. The Quality Assurance Statement(s) will be provided to (b) (4) for inclusion in the final report.

C. Record Retention

All study data, including but not limited to, animal data, formulations data, necropsy data, professional reports, study protocol (including amendments), final report, and any communications concerning the conduct of the study will be retained in the (b) (4) Archive for a period of 5 years following issuance of the final report.

Due to a presumed limited stability, preserved tissues will be maintained for the 1-year period at (b) (4)

Study data generated by the Sponsor or subcontractors will be archived by the Sponsor or subcontractors.

Following the 5-year period (or before at Sponsor's request), the Sponsor will be contacted to determine the disposition of these materials. (b) (4) will maintain all electronic data. (b) (4) will maintain records regarding disposition of data and specimens.

VIII. Proposed Study Timetable

A. Study Initiation Date [a]	See Footnote
B. Experimental Start Date	Date of animal receipt
C. Day of Dosing	February 2, 2007
D. Necropsy	
SD 11:	February 12, 2007
SD 61:	April 3, 2007
SD 91:	May 3, 2007
E. Submission of Audited Draft Final Report	July 5, 2007
F. Study Completion Date [b]	See Footnote

[a] = The date Study Director signs protocol (Protocol must be signed before any study specific procedures are performed).
[b] = The date the report is finalized. The Study Director may finalize the report (by signature) 60 days after submission of the draft final report, if no Sponsor comments are received.

Beth Israel Deaconess (b) (4)

IX. Test and Control Articles

A. Identification and Supplier

1. **Test Article**

Ad26 (b) (4) (a purified, replication-incompetent, recombinant Adenovirus serotype 26 vector that expresses the clade A (b) (4) protein) will be supplied by the Sponsor via Crucell Holland BV. A Certificate of Analysis or equivalent documentation will be provided.

2. **Control Article/Diluent**

Placebo (Formulation Buffer) will be supplied by the Sponsor via Crucell Holland BV. A Certificate of Analysis or equivalent documentation will be provided.

B. Purity and Stability

1. **Test Article**

The purity and stability information for the neat test article will be provided by the Sponsor.

2. **Control Article/Diluent**

Stability can be indicated by a date of expiration in receipt paperwork or other associated documents.

C. Storage Conditions

1. **Test Article**

The adenovirus vaccine will be stored at -75±10°C.

2. **Control Article/Diluent**

The placebo will be stored at 2-8°C.

D. Reserve Samples and Test/Control Article Disposition

1. **Reserve Samples**

Reserve samples (1 vial each) of the neat test and control articles will be taken prior to initial use and will be stored under the same conditions as the neat materials, if reserve samples of the same lot materials have not been taken in other studies. Prior to report finalization, the reserve samples will be returned to Crucell Holland BV for archiving.

2. **Disposition**

Any remaining test and control articles will be returned to the Sponsor, used on subsequent studies, or disposed of at the direction of the Sponsor. Any empty test and control article containers will be disposed of after the final report is issued.

Beth Israel Deaconess (b) (4)

X. Dose Formulations, Sampling and Analysis

A. Formulation

1. **Frequency**

Formulation will not be required at (b) (4) because the control and test articles will be supplied in a ready-to-use form.

2. **Procedure**

Placebo – Remove from refrigerator approximately 30 minutes prior to dosing to let it warm up to room temperature.

Adenovirus vaccine - Thaw at room temperature (18-25°C, on table, not in flow cabinet), which will take approximately 30-45 minutes. After thawing, avoid temperature switches of the material as much as possible (do not transfer the material at 2-8°C or on ice). Start injection as soon as possible after thawing, and the injection needs to be completed within 4 hours after thawing.

3. **Disposition**

Excess formulations will be disposed in accordance with the company's SOPs, appropriate regulatory requirements, and/or information contained in the Material Safety Data Sheets.

B. Dose Formulation Analysis

1. **Formulation Sampling**

Since formulation will not be performed at (b) (4) formulation sampling will not be performed.

2. **Stability**

The date of expiration indicated on the Certificate of Analysis will serve for neat material stability under conditions of storage. Otherwise, data from a stability study or equivalent will be required for inclusion in the final report. Stability should be addressed at -75±10°C and room temperature since these will be the conditions of use for this study.

3. **Dose Analysis**

Dose formulation analysis will not be performed.

XI. Test System and Husbandry

A. Animals

1. **Strain/Source**

New Zealand White Rabbits

Beth Israel Deaconess (b) (4)

(b) (4)

2. **Age at Receipt**

   11 - 16 weeks

3. **Weight at Receipt**

   2-3 kg

4. **Number/Gender**

   48 total; 24 per sex with no more than 3 extras/sex ordered to ensure 48 suitable animals are assigned to study

5. **Identification**

   Individual ear tag and each cage will be labeled with a cage card

6. **Animal Welfare**

   (b) (4) Institutional Animal Care and Use Committee (IACUC) has reviewed this protocol for accordance with provisions of the USDA Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals and the U.S. Interagency Research Animal Committee Principals for the Utilization and Care of Research Animals prior to authorizing its execution.

   In the event of severe toxicity or other life threatening situations in which decisions are to be made regarding treatment or euthanasia of a study animal, the (b) (4) (b) (4) veterinarian and the Study Director will preserve the right for subsequent action.

B. Husbandry

1. **Housing**

   Animals will be individually housed in stainless steel and/or polycarbonate cages.

   Control animals will be housed in a separate room from that of test article-treated animals.

2. **Food**

   Animals will be fed Certified Teklad Global Rabbit Diet #2030, *ad libitum,* except when noted otherwise. Feeders will be changed at least once every two weeks.

   Feed is analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, and organophosphates. General nutrient and contaminant lot release specifications are on file at (b) (4)

Beth Israel Deaconess (b) (4)

3. **Water**

Water is provided *ad libitum* via an automatic watering system and/or water bottles. The water is routinely analyzed for contaminants and specific microbes. The results of these analyses are on file at (b) (4)

4. **Contaminants**

Available information indicates that no substance is present in the diet or drinking water at a concentration likely to influence the outcome of this study.

5. **Environment**

Animals will be housed in a controlled environment (16-22°C and 30-70% relative humidity). Temperature and humidity will be monitored and recorded continuously in each animal room by an environmental monitoring system. In the event of a system failure, manual recording will be performed (once daily) as defined in the Standard Operating Procedures. A 12-hour light/12-hour dark cycle will be maintained except when interrupted by study-related events. These cycle interruptions will be documented in the study data. A minimum of ten air changes/hour will be maintained.

6. **Environmental Enrichment**

Cage enrichment and/or dietary supplements will be provided per the company's SOP.

C. Procedures for All Animals Prior to Randomization

Animals will be acclimated to the facility for at least 7 days prior to the first dose. During that time, animals will be evaluated as shown in **Table 1**. Based on these evaluations, animals considered unsuitable for the study will be excluded from randomization to study groups.

Table 1: Evaluations During Acclimation

Procedure	Frequency
Cageside Observations	≥ 2 daily
Clinical Observations	Prior to Study Day 1
Body Weight	Prior to Study Day 1

Note: Prior to Study Day 1, cageside observations may be recorded by exception.

D. Randomization

Animals will be assigned to study groups using computer generated random numbers. Males and females will be randomized separately. At the time of randomization, the mean body weight for each group will not be statistically different ($p<0.05$) from the control value. Permanent animal numbers will be assigned following randomization.

Beth Israel Deaconess (b) (4)

XII. Study Design

A. Justification for Species, Route of Administration and Dose Levels

This species will be used because of the US FDA recommendation for assessing the biodistribution of adenovirus-vectored vaccines. The intramuscular route was selected since it is the intended route of human exposure. The dose for this study was selected based on the largest amount of vaccine that can be delivered into the rabbit muscle with one injection.

B. Group Designation and Dosage Levels

Table 2: Group Designation and Dosage Levels

Group	Treatment	Dose Level	Dose Volume (mL)	Number of Animals	
				males	females
1	Placebo	0	0.5	9	9
2	Ad26 (b) (4)	0.5 x 10^{11} vp	0.5	15	15

C. Dosing Information

1. **Method of Administration**

   Animals will be dosed via intramuscular injection. Intramuscular injection will be a single 0.5-mL injection into right hind thigh muscle. Dose volume will not be adjusted for body weight. Injection will be administered using needle and syringe. Injection will be administered at a shaved/marked site.

2. **Frequency**

   Once on Study Day (SD) 1. The first day of dosing is designated as SD 1.

3. **Dose Volume**

   Single 0.5-mL injection.

Beth Israel Deaconess (b) (4)

D. Observation of Animals Following Randomization

Table 3: Observation of Animals Following Randomization

Procedure	Frequency of Testing
Cageside Observations [a]	≥ 2 Daily
Clinical Observations [b]	Prior to dose, weekly thereafter, and at termination
Body Weight	Prior to dose, weekly thereafter, and at termination

[a] = Cageside observations will include mortality, moribundity, general health and signs of toxicity.
[b] = Clinical observations will include skin and fur characteristics, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor and behavior patterns.

E. Termination

1. **Unscheduled**

Gross necropsies will be conducted on all moribund animals and all animals not surviving to termination. Moribund animals will be euthanized by sodium pentobarbital or equivalent injection and exsanguinated prior to necropsy (gross necropsy, tissue preservation, histopathology, and bone marrow collection will be performed). Found dead animals will only have gross necropsy, tissue preservation and histopathology performed (no bone marrow collection performed).

2. **Scheduled**

All scheduled animals will be euthanized by sodium pentobarbital or equivalent injection and exsanguinated. Animals will be necropsied as close as possible to the time of sacrifice.

Table 4: Necropsy Schedule

Group	SD 11	SD 61	SD 91
1	3/sex	3/sex	3/sex
2	5/sex	5/sex	5/sex

F. Postmortem Procedures

1. **Gross Necropsy**

Animals will be subjected to a full gross necropsy, which includes examination of the external surface of the body, the injection sites, all orifices, and the cranial, thoracic, and abdominal cavities and their contents.

Beth Israel Deaconess (b) (4)

2. **Bone Marrow Collection**

Unscheduled Sacrifice: Two bone marrow smears will be prepared from the sternum of moribund sacrificed animals. Slides will be air-dried, fixed in methanol, and stored for possible future evaluation. If not evaluated, the slides will be discarded after report finalization.

Scheduled Biodistribution Animals: Bone marrow will be collected from the left femur. The samples will be snap frozen in liquid nitrogen and stored at -75±10°C.

3. **Tissue Collection – Scheduled Biodistribution Necropsy**

The following tissues will be collected in the order listed below with a fresh set of clean instruments for each organ of each animal. Gloves will be changed between each organ. Paired organs will be processed together. The tissues will be snap frozen in liquid nitrogen and stored at -75±10°C. The Group 1 (control) animals will be necropsied first, followed by Group 2 animals.

Blood (≥ 0.6 mL of blood will be collected into an EDTA tube and then transferred to a cryovial and snap frozen)
Ovaries/testis
Liver
Thymus
Heart
Lung
Kidney
Spleen
Mesenteric Lymph Nodes
Iliac Lymph Nodes
Skin and subcutis at injection site
Thigh muscle at injection site
Bone Marrow
Brain

All tissues will be shipped (on dry ice) to (b) (4) and will be processed for the presence of Ad26 (b) (4) in the tissues using a GLP validated method, qPCR (quantitative polymerase chain reaction). Tissues will not be pooled for analysis without prior discussion with the Sponsor. (b) (4) will be responsible for auditing the data generated and will provide a biodistribution report to be included in the final report.

4. **Tissue Collection – Unscheduled Necropsy**

The animal identification and all tissues (sex appropriate) identified to be collected in **Table 5** will be preserved in 10% neutral buffered formalin (NBF). Eye, optic nerve, testis and epididymis will be fixed in modified Davidson's fixative for 24-48 hours and then be transferred to 70% ethanol.

The tissues collected will be preserved for possible histopathologic evaluation. The preserved tissues will be kept at (b) (4) Tissues will be discarded when the

Beth Israel Deaconess (b) (4)

final report is issued following confirmation with the Sponsor and the Study Director.

Table 5: Tissue Preservation List

Tissue	Tissue Collected and Preserved
Adrenal gland	x
Aorta	x
Bone with marrow - femur	x
Bone with marrow - sternum	x
Brain	x
Cecum	x
Cervix	x
Colon	x
Duodenum	x
Epididymis	x
Esophagus	x
Eye	x
Gallbladder	x
Heart	x
Ileum	x
Jejunum	x
Kidney	x
Liver	x
Lung	x
Mammary gland (male and female)	x
Mandibular lymph node	x
Mandibular salivary gland	x
Mesenteric lymph node	x
Optic nerve	x
Ovary	x
Pancreas	x
Parathyroid gland	x
Pituitary	x
Prostate	x
Rectum	x
Sciatic nerve	x
Seminal vesicle	x
Skeletal muscle (biceps femoris)	x
Skin	x
Spinal cord (cervical, thoracic, lumbar)	x
Spleen	x
Stomach	x

Beth Israel Deaconess (b) (4)

Testis	x
Thymus	x
Thyroid gland	x
Tongue	x
Trachea	x
Urinary bladder	x
Uterus	x
Vagina	x
Gross Lesions	x
Injection site[1]	x

[1] Injection site will include underlying muscle.

XIII. Proposed Statistical Analyses

Descriptive statistics (mean, standard deviations, and N) will be presented for all applicable measurement data and shown in the summary tables. Data include but are not limited to:

Body Weight and Body Weight Change

Quantitative results will be analyzed using the Kolmogorov-Smirnov test for normality, the Levene Median test for equal variance and by one-way Analysis of the Variance (ANOVA). If either the normality or equal variance test fails, then the analysis will continue using the non-parametric Kruskal-Wallis ANOVA on rank-transformed data. For parametric data, if the ANOVA indicates statistical significance among experimental groups, then the Dunnett's t-test will be used to delineate which groups (if any) differ from the control. For non-parametric data, if the Kruskal-Wallis ANOVA indicates statistical significance among experimental groups then the Dunn's test will be used to delineate which groups (if any) differ from the control. The probability value of less than 0.05 (two-tailed) will be used as the critical level of significance for all tests.

Statistical analysis will be conducted using SigmaStat™ Statistical Software (b) (4)
(b) (4)

For any group where n=1, no statistical analysis will be performed.

XIV. Final Report

An unaudited and audited draft of the report will be sent to the Sponsor. At finalization, two paper copies (one bound, one unbound) and one electronic copy (PDF) of the final report, which includes the following information, but not limited to, will be submitted to the Sponsor.

Report Table of Contents:

COMPLIANCE STATEMENT

QUALITY ASSURANCE STATEMENT

SIGNATURE PAGE

SUMMARY

Beth Israel Deaconess (b) (4)

Beth Israel Deaconess (b) (4)

(b) (4)

PROTOCOL AMENDMENT

Study Number:	(b) (4)
Study Title:	Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits
Amendment Number:	1

1. **Subject: Section XII.E.2. Scheduled Termination**

   All scheduled animals will be euthanized by intravenous injection of sodium pentobarbital, or Euthasol, or equivalent and exsanguinated.

   Justification: Change to add alternative euthanasia method.

Approval:

(b) (4), (b) (6)
Senior Study Director — Date: 2/12/07

(b) (4), (b) (6)
Sponsor's Representative — Date: 2/9/07

Beth Israel Deaconess (b) (4)

(b) (4)

PROTOCOL AMENDMENT

Study Number:	(b) (4)
Study Title:	Ad26 (b) (4) : 91-Day Intramuscular Single Dose Biodistribution Study in New Zealand White Rabbits
Amendment Number:	2

1. **Subject: Section IV.A. Study Director**

Effective on June 12, 2007, change Study Director to:

(b) (4), (b) (6)

Justification: Study Director is changed due to personnel change (b) (4)

Approval:

(b) (4), (b) (6)

(b) (4), (b) (6) 6-13-07 Date

Study Director

(b) (4), (b) (6)

6/13/07 Date

Sponsor's Representative

(b) (4), (b) (6)

6-13-07

Bridge GPS, Management

CONFIDENTIAL Page 1 of 1 June 12, 2007

(b) (4)

The following deviations from the protocol were noted:

No environmental monitoring data were collected for approximately 11 hours on February 2, 2007.

On several instances throughout the study, the relative humidity was below the protocol-specified lower limit of 30%.

The above-mentioned deviations did not impact this study, nor did they affect the quality or integrity of the study or the interpretation of the results in this report.