Sponsor:	Novartis Vaccines and Diagnostics GmbH & Co. KG
Investigational Product:	Inactivated Tick Born Encephalitis (TBE) virus/strain K 23
Indication:	Prophylaxis against TBE disease
Protocol Number:	V48P2E3
Protocol Title:	A phase IV, randomized, open-label, multi-center study in adults: Evaluation of long-term immunogenicity in subjects boosted with a new TBE vaccine for adults (free of protein-derived stabilizer) in study V48P2E1, 5 years after first booster immunization and Evaluation of booster kinetics in subjects boosted with a new TBE vaccine for adults (free of protein-derived stabilizer), 5 years after first booster immunization.
Phase of Development:	Phase IV
Study Period:	
Date of first enrolment:	17 FEB 2006
Date of last visit:	22 SEP 2006

Methodology:

This was a phase IV, randomized, open-label, multi-center study in adults. All subjects were randomized in a 6:1 ratio into one of the following study groups:

Study Group 1 : Blood draw on Day 0 at year 5 (± 90 days) after first booster immunization in study V48P2E1.

Study Group 2 : Booster vaccination at year 5 (± 90 days) after first booster immunization in study V48P2E1. Additionally blood samples were taken on Day 0, 3, 5, 7, and 21.

All vaccinations were to be conducted at the investigator's site. No off-site vaccination was performed. Subjects who received a booster immunization were observed for 30 minutes after vaccination for any immediate local or systemic reactions. These subjects were instructed to complete a diary card for 4 days (i.e., Booster Day 0 to Booster Day 3) after vaccination to describe local reactions (i.e., erythema, swelling and pain) and systemic reactions (i.e., nausea, malaise, myalgia, arthralgia and headache). During this 4-

day period the body temperature (oral measurement) was also recorded daily, possibly at the same time of the day (preferably in the evening). The use of analgesic/antipyretic medication was also recorded. The diary cards were collected during visit 14, i.e. 5 days after the booster vaccination and data reconciled by the Investigator.

All other adverse events occurring until Day 3 after vaccine administration, and all adverse events leading to a subject's withdrawal from the study or necessitating a physician's visit, and all serious adverse events were collected throughout the study for subjects who received a booster immunization. All adverse events reported during the study were followed until resolution or diagnosis. If an adverse event remained unresolved at the study termination visit, the Investigator and Medical Monitor made a clinical assessment as to whether continued follow-up of the adverse event was warranted.

All prescription medication except minerals and vitamins taken at any time during the study were recorded.

Serum samples for immunogenicity assays (3-5 mL of serum / approx. 10 mL of blood each) were obtained from all subjects at Visit 12 (Day 0 in study V48P2E3) and for subject of study group 2 additionally on Visit 13, 14, 15, 16, (3, 5, 7, 21 days after booster immunization).

For study center 1:

For all subjects 20 mL of blood instead of 10 mL was obtained at Visit 12 (Day 0 in study V48P2E3) and for subject of study group 2 additionally on Visit 13, 14, 15, 16, (3, 5, 7, 21 days after booster immunization), because in the protocol was planned to assess T cell immunity.

Subjects in study group 1 were to be followed for 7 years after first booster immunization in study V48P2E1. This was described in a separate protocol.

Number of Subjects (planned and analyzed):

Up to 179 subjects who had participated in study V48P2E2 were eligible to have a blood draw 5 years (± 90 days) post-booster dose.

Of the original 179, a total of 172 subjects returned for blood draw in study V48P2E3. There were two subjects who were not included in the Enzyme Linked Immunosorbent Assay (ELISA) analysis (who had been vaccinated against yellow fever in 2001, which may influence the results of the ELISA). No information is available about the reasons why the remaining 7 subjects did not return for the V48P2E3 study. The Intention-To-Treat (ITT) population was 172 subjects and the Per protocol (PP) population for the

immunogenicity of the ELISA was 145 and from the analysis based on the Neutralisation Test (NT) was 147.

Study Centers:

Three study centers in Germany.

Publication (reference) and/or ClinicalTrials.gov National Clinical Trial (NCT) Number:

NCT00311493

Objectives:

Primary

1. Descriptive evaluation of the subjects who participated in studies V48P2 (primary immunization), V48P2E1 (first booster immunization) and V48P2E2 (serological follow-up) with respect to antibody titers and percentage of subjects with neutralizing antibodies (NT, in-house, Novartis Vaccines) at 5 years (± 90 days) after the first booster immunization with the new TBE vaccine.
2. To investigate the kinetics of the immune response from Day 0 as defined in protocol V48P2 to year 5 (± 90 days) after first booster immunization with the new TBE vaccine in subjects who were TBE-negative prior to entering V48P2.
3. To investigate the kinetics of the immune response from Day 0 as defined in protocol V48P2 to year 5 (± 90 days) after first booster immunization with the new TBE vaccine in subjects who were TBE-positive prior to entering V48P2.
4. Descriptive evaluation of the subjects who participated in studies V48P2, V48P2E1 and V48P2E2 with respect to antibody titers measured by ELISA (Enzygnost®, Dade Behring).

Secondary

To investigate the kinetics of the immune response from Day 0 as defined in protocol V48P2E3 to Day 3, 5, 7, and 21 for study group 2.

Safety Objectives

To evaluate the subjects for study group 2 with respect to tolerability and safety (using selected local and systemic reactions and adverse event reporting), when boosted with Novartis's TBE vaccine for adults 5 years after first booster immunization in study V48P2E1.

Test Product, Dose, Mode of Administration, Lot Number:

Pre-filled syringes with a total amount of 0.5 mL of TBE vaccine containing 1.5 µg of antigen were provided. Only subjects of study group 2 were vaccinated on Booster Day 0 (2nd booster).

Substance Number:	V48
INN:	TBE-vaccine
Dosage form:	Suspension for Intramuscular injection
Dose:	single dose 0.5 mL containing 1.5µg antigen
Batch No.:	054021G
Expiry Date:	Nov 2006

Duration of Study:

Approximately 10 months overall: 9 months enrollment and 1 – 21 days of individual subject's participation.

It was planned to follow subjects of study group 1 for a total of 7 years following the first booster immunization in order to investigate the kinetics of the immune response to the new TBE vaccine. Blood draws after 7 years was described in a separate protocol.

Reference Therapy, Dose, Mode of Administration, Lot Number:

Not applicable

Statistical Methods:

There is no statistical null hypothesis associated with the immunogenicity objectives, which are analyzed descriptively.

Since there are no statistical hypotheses to be tested, and because of the follow-up nature of this trial, no sample size or power considerations were performed. All available subjects who had participated in study V48P2E2 and who were willing to participate in this study were enrolled; i.e. 179 subjects at maximum.

In a previous trial (BI 71-106/7MN-306IP-DIA; historical vaccine Encepur), 85% of subjects showed seropositive titers (ELISA) 3.5 years after primary immunization with the historical vaccine Encepur. Based on that data a booster immunization was recommended.

Prior to the first booster vaccination (i.e. 1 year after primary immunization), 88% of the subjects showed NT values above or equal to 10 (study V48P2E1).

Diagnosis and Main Criteria for Inclusion and Exclusion:

Inclusion Criteria:

- Healthy volunteers of both sexes aged >18 who participated in study V48P2E2 and were willing to give informed consent.

- Subjects who were available for the duration of the trial (approximately 3 weeks)

- Subjects who were in good health as determined by medical history, physical examination, and clinical judgment of the investigator

Exclusion Criteria:

- Subjects not willing to sign the informed consent form

- Subjects with documented evidence of TBE

- Subjects who received another vaccine within the 4 weeks before the administration of investigational product and 3 weeks after

- Subjects with acute disease at the day of enrollment (acute disease meant moderate or severe illness with or without fever; vaccine could be administered to subjects with minor illness such as mild diarrhea or mild upper respiratory tract infection with body temperature < 38.0°C)

- Subjects with organic brain disturbances, including seizure disorders

- Subjects with progressive neurological disorders

- Subjects who had suffered febrile or afebrile convulsions

- Subjects in whom a general decrease in resistance might have been expected, e.g. those who had recently sustained severe injury or undergone recent surgical operations or in whom surgical operations were planned during the study period, were undernourished, or had disorders involving a decreased immune response

- Subjects being treated with immunosuppressants, systemic corticosteroids for longer than 2 months within the past 4 weeks or during the study period, except for topical or inhaled therapy of mild bronchial asthma

- Subjects being treated with immunoglobulins, whole blood or plasma derivates within the last 3 months and during study participation

- Subjects with autoimmune diseases

- Subjects with evidence of hypersensitivity to the investigational product or chemically related substances in their medical history

- Subjects enrolled in other investigational studies at the same time and within the last 3 months

- Subjects with major congenital defects or serious chronic illness (such as insulin dependent diabetes, cancer, autoimmune diseases) as well as any active severe allergic disease

- Subject with any condition which, in the opinion of the Investigator, might have interfered with the evaluation of the study objectives

- Woman of childbearing age who refused to use an effective method of birth control (abstinence, oral or injected or implanted hormonal contraceptive, diaphragm or condom with spermicidal agent, intrauterine device) beginning 30 days before study entry and continuing through 30 days after the last vaccine dose

Criteria for Evaluation:

Immunogenicity

The long-term immunity of Novartis's TBE vaccine for adults is considered satisfactory if the observed percentage of subjects with neutralizing TBE antibodies as measured by NT (in house, Novartis Vaccines) at 5 years (± 90 days) after first booster immunization is at least 85%.

The kinetic of antibody titers of the booster response is evaluated descriptively.

Safety

Number of subjects with reported local and systemic reactions as well as number of subjects with reported (serious) adverse events (for study group 2 only).

Results:

Table 1. Population Analyzed by Vaccine Group

	Number (Percentages) of Subjects		
	None	**Booster**	**Total**
Total number of subjects enrolled	**N=147**	**N=25**	**N=172**
Enrolled	147 (100%)	25 (100%)	172 (100%)
Safety (Adverse Events)	0	24 (96%)	24 (14%)
Safety (Reactogenicity)	0	24 (96%)	24 (14%)
Full Analysis Set	147 (100%)	25 (100%)	172 (100%)
Per Protocol Set (ELISA)	123 (84%)	22 (88%)	145 (84%)
Per Protocol Set (NT)	125 (85%)	22 (88%)	147 (85%)

Table 2. Summary of Study Terminations, by Vaccine Group - Enrolled Population

	Number (Percentages) of Subjects		
	None	**Booster**	**Total**
Total number of subjects enrolled	**N=147**	**N=25**	**N=172**
Completed protocol	144 (98%)	24 (96%)	168 (98%)
Premature withdrawal	3 (2%)	1 (4%)	4 (2%)
Withdrawal of consent	0	1 (4%)	1 (<1%)
Inappropriate enrollment	3 (2%)	0	3 (2%)

Table 3. Demography and Baseline Characteristics by Vaccine Group – Enrolled Population

	Number (Percentages) of Subjects		
	None	**Booster**	**Total**
Total number of subjects enrolled	**N=147**	**N=25**	**N=172**
Age (Years):	38.0±7.8	37.2±7.1	37.8±7.7
Sex			
Male	71 (48%)	15 (60%)	86 (50%)
Female	76 (52%)	10 (40%)	86 (50%)
2nd Booster After Termination of V48P2E2?			
No	147 (100%)	25 (100%)	172 (100%)
Meeting Entry Criteria?:			
Yes	144 (98%)	25 (100%)	169 (98%)
No	3 (2%)	0	3 (2%)

Table 4. Immunogenicity, Determined by NT, in the PP and ITT Populations, After Booster

GMTs, GMRs and Percentages of Subjects with Neutralizing Antibodies and Seroconversion/ Significant Increase (95% CIs) after Booster					
	Timepoint	**PP Data**	**ITT Data**		
		TBE negative N=147	**TBE negative N=159**	**TBE positive N=13**	**ITT Total N=172**
GMT (95% CI)	Baseline [a] Day 0	1 (1-1)	1 (1-1)	17 (8.94-34)	1.25 (1.1-1.42)
	Pre-Booster [b]	37 (31-44)	37 (31-44)	229 (92-573)	42 (35-51)
	Booster + 21 days [b]	1617 (1308-2000)	1609 (1315-1967)	446 (192-1036)	1460 (1195-1784)
	Booster + 3 years [c]	309 (254-376)	293 (242-364) [e]	185 (91-378)	283 (236-340) [f]
	Booster + 5 years [d]	308 (253-375)	297 (246-359)	185 (94-365)	287 (239-344)
GMR (95% CI)	Booster + 5 years [d]/ Pre-Booster [b]	-	-	-	6.87 (5.43-8.46)

	Booster + 5 years [d]/ Booster + 21 days [b]	-	-	-	0.2 (0.17-0.23)
	Booster + 5 years [d]/ Booster + 3 years [c]	-	-	-	1 (0.9-1.11)
% (95% CI)	NT ≥ 2 [d] at 5 years after booster	100% (98%-100%)	100% (98%-100%)	100% (75%-100%)	100% (98%-100%)
	NT ≥ 10 [d] at 5 years after booster	99% (96%-100%)	99% (97%-100%)	100% (75%-100%)	99% (97%-100%)

[a] = Data from V48P2 for population enrolled in V48P2E3 (Visit 1); [b] = Data from V48P2E1 for population enrolled in V48P2E3 (Visit 6: pre-booster; Visit 7: 21 days post-booster); [c] = Data from V48P2E2 for population enrolled in V48P2E3 (Visit 10); [d] = Data from V48P2E3 for population enrolled in V48P2E3 (Visit 12); [e] = N=157; [f] = N=170

Table 5. GMTs Determined by NT of the PP Population (All Seronegative at Visit 1) and ITT Population (Seronegative at Visit 1) and GMRs (Visit 12 / Visit 7 to 11) of the Overall ITT Population (Seropositive and Seronegative)

	GMT (95% CI) PP	**N**	**GMT (95% CI) ITT**	**N**	**GMR: Visit 12/Visit X [c] (95% CI)**
Visit 1[a] (Day 0)	1 (1-1)	147	1 (1-1)	159	-
Visit 4 [a] (Day 42)	47 (40-54)	147	46 (39-53)	159	-
Visit 5 [a] (Day 56)	43 (37-48)	147	43 (38-49)	159	-
Visit 6 [a] (Pre-booster)	37 (31-44)	147	37 (31-44)	159	6.78 (5.43-8.46)
Visit 7 [a] (Booster + 21 days)	1617 (1308-2000)	147	1609 (1315-1967)	159	0.20 (0.17-0.23)
Visit 8 [a] (Booster + 1 year)	455 (372-558)	143	437 (360-531)	154	0.68 (0.59-0.78)
Visit 9 [a] (Booster + 2 years)	382 (313-468)	145	369 (304-447)	156	0.82 (0.74-0.92)
Visit 10 [a] (Booster + 3 years)	309 (254-376)	147	293 (242-354)	157	1 (0.9-1.11)
Visit 11 [a] (Booster + 4 years)	367 (300-450)	141	358 (296-434)	153	0.83 (0.74-0.93)
Visit 12 [b] (Booster + 5 years)	308 (253-375)	147	297 (246-359)	159	-

[a] = Data from preceding studies for population enrolled in V48P2E3; [b] = Data from V48P2E3; [c] = Providing GMRs of Visit 12 to baseline Visits 6-11 as defined in the left column for the overall ITT population

Table 6. GMTs Determined by NT of Subjects Seropositive at Visit 1; ITT Population.

	GMT (95% CI)	N
Visit 1[a] (Day 0)	19 (8.04-45)	13
Visit 4 [a] (Day 42)	803 (411-1569)	13
Visit 5 [a] (Day 56)	772 (433-1375)	13
Visit 6 [a] (Pre-booster)	229 (92-573)	13
Visit 7 [a] (Booster + 21 days)	446 (192-1036)	13
Visit 8 [a] (Booster + 1 year)	239 (115-496)	13
Visit 9 [a] (Booster + 2 years)	150 (92-243)	12
Visit 10 [a] (Booster + 3 years)	185 (91-378)	13
Visit 11 [a] (Booster + 4 years)	212 (105-428)	12
Visit 12 [b] (Booster + 5 years)	185 (94-365)	13

[a] = Data from preceding studies for population enrolled in V48P2E3; [b] = Data from V48P2E3

Table 7. GMCs (U/mL), GMRs and Percentages of Subjects with ELISA TBE Antibodies Above the Seroconversion Limit (95% CIs), After Booster

GMTs, GMRs and Percentages of Subjects with Neutralizing Antibodies and Seroconversion/ Significant Increase (95% CIs) After Booster					
	Timepoint	**PP Data**	**ITT Data**		
		TBE negative N=145	**TBE negative N=159**	**TBE positive N=13**	**ITT Total N=172**
GMC (95% CI)	Baseline [a] Day 0	3.29 (3.2-3.39)	3.29 (3.2-3.38)	31 (14-68)	3.9 (3.5- 4.33)
	Pre-Booster [b]	14 (12-16)	14 (12-17)	156 (97-250)	17 (15-20)
	Booster + 21 days [b]	223 (198-251)	226 (201-255)	308 (249-380)	232 (207-259)
	Booster + 3 years [c]	66 (57-77)	65 (56-74)	119 (69-207)	68 (59-78)
	Booster + 5 years [d]	66 (57-75)	66 (58-75)	138 (78-244)	69 (61-79)
GMR (95% CI)	Booster + 5years [d]/ Pre-Booster [b]	-	-	-	4.05 (3.45-4.76)
	Booster + 5years [d]/ Booster + 21 days [b]	-	-	-	0.3 (0.27-0.33)
	Booster + 5years [d]/ Booster + 3 years [c]	-	-	-	1.01 (0.96-1.07)
% with TBE titers above seroconversion limit (95% CI) 5 years after booster		97% (93%-99%)	97% (93%-99%)	100% (75%-100%)	97% (93%-99%)

[a] = Data from V48P2 for population enrolled in V48P2E3 (Visit 1); [b] = Data from V48P2E1 for population enrolled in V48P2E3 (Visit 6: pre-booster; Visit 7: 21 days post-booster); [c] = Data from V48P2E2 for population enrolled in V48P2E3 (Visit 10); [d] = Data from V48P2E3 for population enrolled in V48P2E3 (Visit 12)

Table 8. GMTs (95% CIs) in the PP and ITT Population and GMRs in the ITT Population, up to 21 Days After Second Booster

	Timepoint	PP (N=22)	ITT (N=25)
GMT	Pre booster (Visit 12)	378 (233-615)	323 (201-520)
	Booster + 3 days (Visit 13)	326 (204-519)	315 (205-482)
	Booster + 5 days (Visit 14)	355 (220-572)	336 (215-524)
	Booster + 7 days (Visit 15)	579 (364-920)	518 (330-813)
	Booster + 21 days (Visit 16)	1366 (893-2089)	1382 (939-2036)
GMR	Visit 13/Visit12	-	0.88 (0.76-1.02)
	Visit 14/Visit12	-	0.94 (0.8-1.11)
	Visit 15/Visit12	-	1.46 (1.1-1.92)
	Visit 16/Visit12	-	3.89 (2.72-5.55)

Table 9. Overview of Solicited Adverse Events after Booster, Days 0-3

	Total(N=24)
With Any Postinjection Reaction	22 (92%)
With Any Local Reactions	21 (88%)
With Any Systemic Reactions	13 (54%)
With Other* Postinjection Reactions	5 (21%)

* ”other” postinjection reactions: use of analgesic/antipyretic medication

Table 10. Number of Subjects with Local Reactions

		Number of subjects (Percentages)
Erythema	> 0 –25 mm[1]	9 (38%)
	10 –25 mm[2]	0
	> 25 – 50 mm	1 (4%)
	> 50 mm	0
Swelling	> 0 –25 mm[1]	2 (8%)
	10 –25 mm[2]	0
	> 25 – 50 mm	2 (8%)
	> 50 mm	1 (4%)
Pain	Mild	14 (58%)
	Moderate	6 (25%)
	Severe	1 (4%)

[1] erythema and swelling classification scheme I: none (i.e., = 0 mm), >0 to 25 mm, >25 to 50 mm and >50 mm; [2] erythema and swelling classification scheme II: none (i.e., <10 mm), 10 to 25 mm, >25 to 50 mm and >50 mm.

Table 11. Number of Subjects with Systemic Reactions

	Number of subjects (Percentages)			
	mild	**moderate**	**severe**	**any**
Malaise	9 (38%)	0	0	9 (38%)
Myalgia	7 (29%)	0	0	7 (29%)
Arthralgia	1 (4%)	0	0	1 (4%)
Nausea	0	1 (4%)	0	1 (4%)
Headache	3 (13%)	4 (17%)	1 (4%)	8 (33%)

Table 12. Overview of Unsolicited Adverse Events

	Number of subjects (Percentages)
	Total (N=24)
With any other adverse events	4 (17%)
With at least possibly related AE	3 (13%)
Serious Adverse Events	0

Table 13. Serious Adverse events by Preferred Term Sorted by System Organ Class

None Reported

Table 14. Adverse Events Reported by ≥ 5% of Subjects by Preferred Term Sorted by System Organ Class

MedDRA System Organ Class	**Number (Percentages) of Subjects**[1]
MedDRA Preferred **Term**	**Booster (N=24)**
Gen. Disorders & Admin. Site Cond.	3 (13%)
Injection Site Pain	3 (13%)
Injection Site Swelling	2 (8%)
Infections & Infestations	2 (8%)
Nasopharyngitis	2 (8%)

[1]Number and percent of subjects with one or more events (as reported on Adverse Events form) that map to MedDRA preferred term. Hence, MedDRA preferred term counts may not sum to overall counts.

Conclusion:

Based on the aforementioned results, prolongation of the booster interval from 3 to 5 years after the first booster immunization is justified. There is evidence that titers will remain on a high and protective level for more than 5 years.

The second booster immunization with the polygeline-free TBE vaccine 5 years after the first booster appeared to be well tolerated by all subjects. No unexpected or serious adverse events were reported. Therefore, no safety concerns for the booster immunization with the polygeline-free TBE vaccine were found in this phase IV study.

Date of Clinical Trial Report: 25 MAY 07