Statistical Analysis Plan	
Test Product	Revacept
EudraCT number	2011-001006-10
Protocol	Revacept/CS/02 22.06.2015 (Version 8)

Revacept, an inhibitor of platelet adhesion in symptomatic carotid stenosis: A phase II, multicentre, randomised, dose-finding, double-blind and placebo-controlled superiority study with parallel groups

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Study period:

Early 2012 – End of 2019

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List of Abbreviations

AE	Adverse event
ANCOVA	Analysis of Covariance
BP	Blood pressure
CEA	Carotid endarterectomy
CI	Confidence Interval
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
ICA	Internal carotid artery
ICH	International Conference of Harmonisation
ITT	Intention-To-Treat
MES	MicroEmbolic Signals
MTD	Maximum tolerated dose
PP	Per Protocol
PT	Preferred Term
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SOC	System Organ Class
TCD	TransCranial Doppler
TIA	Transient Ischaemic Attack
WOCBP	Women of childbearing potential

Statistical Analysis Plan
Approval Form

The Statistical Analysis Plan has been reviewed and approved by the following signatories:

Biostatistician (d.s.h.)

Signature: _____ Date: _____

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Sponsor:

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1. OVERVIEW

This statistical analysis plan (SAP) provides a comprehensive and detailed description of the strategy, rationale, and statistical techniques to be used for in the assessment of the efficacy and safety of Revacept in subjects with extracranial carotid artery stenosis and thrombotic secondary complications such as microembolic signals (MES) and microinfarctions in the brain (DWI-NMR). This SAP provides additional details concerning the statistical analyses outlined in the protocol Revacept/CS/02 (22.06.2015).

A blind review of the data shall be performed before the final analysis within the framework of the requirements of the ICH Guideline E9. The Blind Review Report will include the final statistical analysis plan (updated statistical analysis plan) and the final definition of data sets. If the blind review suggests changes to the principal features stated in the protocol, either a formal protocol amendment (in case of a major change, e.g. change in the definition of a primary endpoint) will be issued or it will be documented in the SAP and the Clinical Study Report.

The Blind Review Report will be finalized before the blind is broken at the stage of the interim and/or final analysis. Formal records shall be kept of when the statistical analysis plan was finalized as well as when the blind was subsequently broken.

2. STUDY OBJECTIVES

The efficacy endpoints are:

- to evaluate whether the incidence of preoperative microembolic signals (MES) is reduced in patients with symptomatic carotid artery stenosis who have been treated with Revacept plus antiplatelet therapy (aspirin or clopidogrel) versus antiplatelet therapy-therapy alone (placebo) prior to carotid endarterectomy (CEA) or carotid artery stenting. MES will be assessed by transcranial Doppler (TCD) examination (before and after treatment).
- Rate of MES per hour (before and after treatment)
- Assessment of neurological status (NIH Stroke Scale)
- Cerebral lesion analysis by DWI-NMR and correlation to neurological status (before and after treatment and CEA or stenting)
- Clinical endpoints will be summarised cumulatively i.e. before treatment, one and three days after treatment, at 3 months and at 12 months. The following endpoints will be recorded:
  - Rate of all cause death
  - Rate of stroke-related death
  - Any TIA or stroke including haemorrhagic stroke
- Assessment of cardiovascular outcome including myocardial infarction and re-intervention up to 3 and 12 months
- Combined clinical endpoints for ischemic events (myocardial infarction and ischemic stroke/TIA) and rate of rescue medication (additional anti-platelet co-medication) during the study

Safety will be summarised by treatment group and include the following:

- Vital signs
- ECG parameters
- Anti-drug antibody titres
- Reporting AEs including wound healing complications, laboratory abnormalities and use of concomitant medication
- Haemostasis will be closely monitored by assessing:
  - laboratory parameters indicating thrombocytopenia and bleeding according to the RELY study group criteria
  - where feasible: in vitro platelet function with collagen, TRAP and ADP-mediated platelet aggregation and in vitro bleeding time by PFA-100 / PFA-200

3. STUDY DESIGN

Number of centers:	16 active centers (planned ≥3 centers)
Randomized:	Yes
Blinded:	double-blinded
Design:	Phase II study
Dosing:	Dose-finding - 2 doses
Placebo controlled:	Yes
Strata:	Yes - anti-platelet therapy prior to screening - statin therapy prior to screening - degree of carotid stenosis
Treatments:	Placebo 40 mg Revacept 120 mg Revacept

3.1 Assigning Subjects to Treatment Groups / Randomisation

Eligible subjects were randomised to one of three treatment groups.

The first 10 patients were treated sequentially, e.g. only one patient was randomised at a time. The next patient was randomised only once the first patient has completed visit 5 and and not suffered a suspected unexpected serious adverse event. Once the first 10 patients have completed the sequential phase and the DSMB has recommended continuing the trial, parallel recruitment was started (see also section 1.3 of the protocol).

The study was conducted at 16 study centres which were planned to screen and recruit 150 eligible patients. During the recruitment period, each study centre was expected to provide the capacity and willingness to recruit at least 20 patients. There was a competitive recruitment of patients, which means that some hospitals were to enrol more than 20 patients and some less.

However, if a study centre randomised less than 3 patients, further recruitment of patients may have been stopped because the quality of a study was dependent on the number of patients per centre. Very few patients in a centre might be related to a higher rate of missing data or patient visits and a higher withdrawal rate.

Patients were assigned to a treatment group by web-based online randomisation. Each patient was allocated to one of the three treatment arms using a minimised randomisation method in order to balance potential prognostic factors between individual treatment arms. The following stratification factors had impact on treatment allocation:

(1) Patient has received anti-platelet therapy with aspirin or clopidogrel prior to screening (Yes/No)
(2) Patient has received statin therapy prior to screening (Yes/No)
(3) Degree of carotid stenosis (50 - 70% / > 70%)

3.2 Blinding

Emergency unblinding is performed using the web-based online randomisation tool. The Investigator should ensure that the code is broken only in accordance with the protocol. For unblinding, the Investigator should note the date, time and reason for unblinding in the patient notes and promptly inform the Monitor or Sponsor and the Coordinating Investigator of any premature unblinding (e.g. accidental unblinding, unblinding due to a serious adverse event) of the investigational product(s).

3.3 Sample size

Primary sample size estimation was conducted based on the CARESS study results (Markus et al., 2005). 50 patients should be allocated to each treatment arm. Due to low incidence of MES (high screening failure rates) study design was changed to exploratory study (Version 8 of the study protocol).

4. STUDY VARIABLES

4.1 Efficacy endpoints

- to evaluate whether the incidence of preoperative microembolic signals (MES) is reduced in patients with symptomatic carotid artery stenosis who have been treated with Revacept plus antiplatelet therapytherapy (aspirin or clopidogrel) versus antiplatelet therapytherapy alone (placebo) prior to carotid endarterectomy (CEA) or carotid artery stenting.
  MES was assessed by transcranial Doppler (TCD) examination (before and after treatment).
- Rate of MES per hour (before and after treatment)
- Assessment of neurological status (NIH Stroke Scale)
- Cerebral lesion analysis by DWI-NMR and correlation to neurological status (before and after treatment and CEA or stenting)
- Clinical endpoints will be summarised cumulatively i.e. before treatment, one and three days after treatment, at 3 months and at 12 months. The following endpoints will be recorded:
  - Rate of all cause death
  - Rate of stroke-related death
  - Any TIA or stroke including haemorrhagic stroke
- Assessment of cardiovascular outcome including myocardial infarction and re-intervention up to 3 and 12 months
- Combined clinical endpoints for ischemic events (myocardial infarction and ischemic stroke/TIA) and rate of rescue medication (additional anti-platelet co-medication) during the study

4.2 Safety endpoints:

- Vital signs
- ECG parameters
- Anti-drug antibody titres
- Reporting AEs including wound healing complications, laboratory abnormalities and use of concomitant medication
- Haemostasis will be closely monitored by assessing:
  - laboratory parameters indicating thrombocytopenia and bleeding according to the RELY study group criteria
  - where feasible: in vitro platelet function with collagen, TRAP and ADP-mediated platelet aggregation and in vitro bleeding time by PFA-100 / PFA-200

5. STUDY PLAN

The study schedule was as follows:

- Visit 1: Screening
  Unsuccessfully screened subjects may have been re-screened if necessary. Visit 1 procedures should have been performed within 24 hours.

- Randomisation
  Once all screening examination results were available and indicated that the patient was eligible for the study, patients were allocated to a treatment arm by stratified randomization.

- Visit 2: Treatment
  Pharmacokinetic blood sampling (for approximately 20 % of patients)
  - t0 prior to IMP administration
  - t0.5h 30 mins (± 5 mins) after start of IMP infusion
  - t6h 6 h (± 1 hr) after start of IMP infusion

  Administration of study medication was performed by intravenous infusion for 20 minutes.

- Visit 3: 24 hrs after treatment (between 2 hrs and 46 hrs after treatment)
  Pharmacokinetic blood sampling (for approximately 20 % of patients)
  - t24h 24 hours (± 4 hrs) after start of IMP infusion

- Visit 4: CEA / intervention (3 days after treatment, -69 hrs / + 5 days)
  Pharmacokinetic blood sampling (for approximately 20 % of patients)
  - t3d 3 days (± 48 hrs) after start of IMP infusion

- Visit 5: 24 hrs after CEA or other intervention (± 24 hrs)

- Visit 6: follow up 3 months after treatment (± 1 month)
  Pharmacokinetic blood sampling (for approximately 20 % of patients)
  - t3m 3 months (±1 month) after IMP infusion

- Visit 7: 12 months follow up (± 1 month)
  Assessment of clinical outcome

		Screening	Randomisation	Treatment (T)	T +24 hrs (±22 hrs)	CEA T + 3 d (-69 hrs/+5d)	CEA + 24 hrs (±12 hrs)	Follow Up	
								T+ 3 m (±1 m)	T + 12 m (±1 m)
Procedure	**Visit**	1	-	2	3	4	5	6	7
Informed consent		x							
Randomisation			x						
Study medication (Revacept or placebo)				x					
CEA or other intervention						x[r]			
CEA / intervention outcome							x[r]		
Assessment of wound healing Complications							x[r]	x	
Anamnesis		x							
Concomitant medication		x		x	x	x	x	x	
Physical examination		x[r]		x	x	x[r]	x[r]	x	
Adverse events				x	x	x	x	x	
MRS, Barthel Index				x[r]				x	
NIH Stroke Scale				x[r]			x[r]	x	
Clinical outcome								x	x
TCD		x			x				
Electrocardiogram		x[r]			x		x[r]	x	
DWI-NMR		X[a,r]					x		
Laboratory Tests	Biochemistry	x[r]			x[r]		x[r]	x	
	Haematology / Bleeding	x[r]			x[r]		x[r]	x	
	Coagulation	x[r]			x[r]		x[r]	x	
	Urinalysis	x[r]			x[r]		x[r]	x	
	In vitro bleeding time (PFA100) and aggregation			X*	X*	X*		X*	
	Pregnancy test	x							
	Pharmacokinetics (selected patients)			x[b]	x[c]	x[c]		x[e]	
	Anti-drug antibodies			x				x	

[r]routine assessment / blood sampling
* where feasible
[a]eligible patients only
[b]drawing times: t0 prior to IMP administration, t 0.5h 30 mins (±5 mins) after start of IMP infusion, t 6h: 6 h (±1 hr) after start of IMP infusion
[c]drawing time: t24h (±4 hrs) after start of IMP infusion
[d]drawing time: t3d (±48hrs) after start of IMP infusion
[e]drawing time: t3m (±1month) after start of IMP infusion

6. ANALYSIS SETS

6.1 Safety Set

All Subjects who have received study medication and have had one contact with the Investigator afterwards will be analysed for safety.

6.2 Full Analysis Set

For efficacy, all subjects who have had at least one dose of medication will be included in the ITT analysis. The ITT analysis is defined as the first line efficacy analysis.

6.3 Per Protocol Set

The PP population includes all subjects who are eligible for ITT evaluation and who, in addition, do not show major protocol deviations.

Exclusion criteria for the study are:

- Extracranial carotid artery stenosis (diagnosed by vascular duplex ultrasound peak flow or angiography) - Lesions with < 50 % stenosis according to European Carotid Surgery Trial (ECST) criteria
- NIHSS score > 18
- Recent intracerebral haemorrhage by X-ray computed tomography (CT) or nuclear magneticresonance (NMR)
- Cardiac cause of embolisation (atrial fibrillation or other cardiac source e.g. artificial heart valves)
- History of hypersensitivity, contraindication or serious adverse reaction to inhibitors of platelet aggregation, hypersensitivity to related drugs (cross-allergy) or to any of the excipients in the study drug
- History or evidence of thrombocytopenia (<30.000/µl), bleeding diathesis or coagulopathy (pathological international normalised ratio (INR) or activated partial thrompoplastin time (aPTT))
- Thrombolysis within the last 48 hours
- Relevant haemorrhagic transformation as determined by CT or anamnesis
- Oral anticoagulation or dual anti-platelet therapy within aspirin or clopidogrel and other P2Y inhibitor at screening (3 days for dipyridamole extended release; 8 hours for tirofiban/Aggrastat)
- Sustained hypertension (systolic BP >179 mmHg or diastolic BP >109 mmHg), hypertensive patients shall be treated in accordance with current guidelines for the management of arterial hypertension
- History of severe systemic disease such as terminal carcinoma, renal failure (or current creatinine >200 µmol/l), cirrhosis, severe dementia, or psychosis

- Current severe liver dysfunction (transaminase level greater than 5-fold over upper normal range limit)
- Active autoimmune disorder such as systemic lupus erythematosus, rheumatoid arthritis,vasculitis or glomerulonephritis
- Known atrial fibrillation or other clinically significant ECG abnormalities (at present)
- Acoustic window that does not allow for TCD recording
- Subjects who are compulsorily detained for treatment of either a psychiatric or physical illness (e.g. infectious disease)
- Ongoing drug or alcohol abuse

7. STATISTICAL CONSIDERATIONS AND DEFINITIONS

7.1 Statistical Software

All statistical analyses will be performed using the SAS® system (Version 9.4).

7.2 Protocol Deviations and Violations/Data Review

After all queries issued and answered to the extent possible, all medical codes are approved, all SAEs are reconciled, and prior to locking and unblinding the database, a Data Review Meeting will be held. The primary purpose of the meeting will be to resolve outstanding data issues, to examine protocol deviations, to define the analysis sets, and to finalize other statistically related issues.

At the Blinded Data Review Meeting, possible protocol violations will be classified as “major”, “minor“, or “not a protocol violation”. Subjects will be allocated to the individual data sets with regard to the classification of possible protocol violations. The final data sets shall be described in detail in the Blinded Data Review Report.

Major protocol deviations - leading to exclusion from the PP population - are - but not limited to - any of the following conditions:

- Patient did not receive the study medication as planned
- Oral anticoagulation or dual anti-platelet therapy within aspirin or clopidogrel and other P2Y inhibitors at screening (3 days for dipyridamole extended release; 8 hours for tirofiban/Aggrastat)
- Other concomitant medication prohibited according to the exclusion / inclusion criteria
- Systolic blood pressure >190 mmHg
- Cardiac cause of embolisation (atrial fibrillation or other cardiac cause e.g. artificial heart valves)

Definitions of protocol deviations checked by program are summarized in the following table. This table also includes proposals for classifying protocol deviations as “major”, which are referred to as protocol violations. These should be seriously considered and discussed during the

data review meeting with regard to their impact on statistical analysis and the validity of the results, and the definition of subject populations.

Table 6-1: Definitions of protocol deviations

No.	Criterion	Major deviation (violation)
1	Any of the inclusion criteria answered with 'No' Answers will be checked by other sources in the CRF - if possible	Yes or otherwise declared in the definitions in blind review report
2	Any of the exclusion criteria answered with 'Yes' Answers will be checked by other sources in the CRF - if possible	Yes or otherwise declared in the definitions in the blind review report
3	Patient did not receive the study medication as planned	Yes

7.3 Handling of Drop-outs or Missing Values

Missing values for the primary analyses at Visit 3 respectively 5 will be imputed by the last observation carried forward approach. This means patients who dropped out before Visit 3 respectively 5 will be counted as non-responder.

7.4 Multicentre Analysis

The analysis of combined centers is the primary analysis of efficacy endpoints. A meta-analysis approach will be chosen to pool the center dependent results for statistical analysis and to investigate for heterogeneity in the strata (e.g. Whitehead, 2003).

7.5 Multiple Comparison

As this was an exploratory study no confirmatory hypotheses have to be tested

7.6 General Calculation Rules

Binary, categorical and ordinal parameters will be summarised by means of absolute and percentage numbers (including ‘missing data’ as valid category at visit 1). Numerical data will be

summarised by means of standard statistics (i.e. number of available data, mean, standard deviation, minimum, median, maximum, lower and upper quartile). Wherever useful, the summary statistics will be presented by stratification according to centers and/or visit.
In addition, adequate figures (e.g. bar charts, Box-Whisker-Plots) will be presented to summarise the results for some parameters.

All statistical tests will be performed two-sided and will be interpreted in a descriptive-exploratory way. Two-sided confidence intervals (CIs) will be displayed for important variables. Appropriate methods will be used to derive confidence intervals, depending on data nature and distribution. All safety and tolerability data will be presented in a purely descriptive manner as outlined below.

8. STATISTICAL ANALYSIS

8.1 Disposition of Subjects and Withdrawals

Patients without any documented application of any study medication (without any documented infusion) will be excluded from all statistical analyses, but will be displayed in all data listings.

- The disposition of patients with regard to
  - enrolment of 1st patient and last follow-up of last patient
  - the number of patients enrolled (=subjects who gave informed consent),
  - number of study centres
  - number of enrolled patients by study centre
  - the number and percent of patients who discontinued prematurely within the study period,
  - the number and percent of patients who completed the study by treatment group and study centres.

  will be summarised by means of frequency tables.
- For patients with a premature discontinuation the frequency of reason for discontinuation due to
  - AE
  - SAE
  - Withdrawal of consent
  - Investigator decision
  - Death
  - Termination criterion arose: Specification
  - other

  will be summarised by treatment group and study centres.

In addition, all treated patients who discontinued prematurely from the study treatment and/or the study (according to the corresponding CRFs) will be listed; at least the time-point of discontinuation and the main reason for discontinuation.

- Analysis population

  Inclusion/Exclusion criteria will be listed in the individual listing of raw data

  Adherence to the protocol will be described by

  - number and percent of patients with at least one deviation from the protocol
  - number and percent of patients with at least one major deviation from protocol
  - number and percent of patients with major deviations from protocol by type of protocol deviation
  - number and percent of patients adhering to each analysis population

  using frequency tables

8.2 Demographics and Baseline Characteristics

Demographic data and all other important characteristics assessed at the Screening visit (Visit 1) will be presented for the Safety, Intention-To-Treat and Per-Protocol analysis set. The results will be presented in a descriptive way.

Laboratory assessments at the Screening visit will be evaluated in the Safety analysis section.

8.2.1 Demographics

Demographic variables will be evaluated from the Screening assessment (Visit 1).

- Continuous demographic variables (age, height, weight) will be summarized using usual descriptive statistics.
- Nominal data (ethnic origin, gender and hormone status) and ordinary data (age classes) will be summarized by means of absolute and percent numbers.

A homogeneity analysis will be performed for the ITT and PP population.

8.2.2 Baseline characteristics – Anamnesis

The following categorical baseline data will be analyzed descriptively by means of absolute and percent numbers

- Medical history
  Pre-existing diseases including surgeries/interventions for the last 3 months will be described presenting

  - number and percent of patients with previous diseases or surgeries if documented
  - frequency distribution of diseases and surgeries as reported by the investigator

  and will be summarized by means of frequency tables.

A homogeneity analysis will be performed for number of patients with previous diseases or surgeries for the ITT and PP population using statistical tests as appropriate.

8.2.3 Baseline characteristics – Physical examinations

- Results of physical examination, assessment of
  - heart (normal/abnormal)
  - lung (normal/abnormal)
  - abdomen (normal/abnormal)
  - oedema (normal/abnormal)

will be described presenting number and percent of patients with abnormal findings and summarized by means of frequency tables.

A homogeneity analysis will be performed for physical assessment (using Fisher's exact test) for the ITT and PP population.

8.2.4 Previous and concomitant medication

The following classification of medication taken for the last 3 months will be performed:

a) Previous medication - discontinued before start of treatment
b) Previous medication - continued at start of treatment (infusion)

Previous and concomitant medication will be described presenting

- number and percent of patients
- type of medication (as reported)
- type of medication (ATC4 level) by indication (Preferred Term)

and summarized by means of frequency tables.

A homogeneity analysis will be performed for number of patients with previous and concomitant medication of categories a) and b) using Fisher's exact test for the ITT and PP population.

8.2.5 Risk factors

The following risk factors were assessed at the Screening visit:

- Blood pressure

  will be summarized by means of descriptive statistics.

- Physical exercises >30 min/week

  will be summarized by means of frequency tables

- Laboratory values
  - Glucose (fasting) [mg/dL]
  - HbA1c [mg/dL]
  - Cholesterine [mg/dL]
  - HDL [mg/dL]
  - LDL [mg/dL]
  - Triglyceride [mg/dL]

  will be summarized by means of descriptive statistics

- Smoking habits
  - Type of smoker (smoker, ex-smoker, non-smoker)
  - Smoking period (duration in years)
  - Number of cigarettes per day with the classes <5 cigarettes, 5-<10 cigarettes10-<20 cigarettes, 20-<30 cigarettes, 30-<40 cigarettes, 40-<50 cigarettes, >50 cigarettes, other

  will be summarized by means of frequency tables

- Alcohol consumption

  consumption of

  - beer with the classes (none,<250mL, 250-500mL,500mL-1000mL,>1000mL,other)
  - wine with the classes (none,<100mL, 100-250mL,>250mL,other)
  - spirits with the classes (none,<10mL,10-20mL,>20mL,other)

  will be summarized by means of frequency tables

A homogeneity analysis will be performed for the risk factor parameters using Fisher's exact test, Wilcoxon test and Mantel-Haenszel test for the ITT and PP population.

8.2.6 Bleeding

The following assessments of bleeding will be evaluated exploratory using descriptive methods of statistics.

- Reduction in haemoglobin level
  - by at least 20 g/L
  - by at least 50 g/L
- Requiring transfusion
  - of ≥2 units of blood
  - of ≥4 units of blood
- Symptomatic bleeding in a critical area of organ
  - Intra-ocular
  - Intra-spinal
  - Intra-musculär
  - Retro-peritoneal
  - Intra-articular
  - Pericardial

  - Subdural intra-cranial
  - Intra-cerebral
- Other symptomatic bleeding
  - Gastrointestinal
  - Other organ
- Involving hypotension
- Surgery necessary
- Fatal

A homogeneity analysis of bleeding parameters will be performed using Fisher's exact test for the ITT and PP population.

8.2.7 Electrocardiography

Parameters examined are heart rate, type of rhythm, clinically significant ST-segment deviation and interval times.

- Heart rate [beats/minute]

  will be summarized by means of descriptive statistics.

- Type of rhythm
  - Sinus rhythms
  - Atrial fibrilation
  - Other rhythm

  will be summarized by means of descriptive statistics.

- Clinically significant ST-segment deviation

  will be summarized by means of descriptive statistics.

- Interval times
  - P [ms]
  - PQ interval [ms]
  - QRS complex [ms]
  - QTc [ms]

  will be summarized by means of descriptive statistics.

A homogeneity analysis will be performed for the parameter heart rate using the Wilcoxon test, type of rhythm using the Mantel-Haenszel test and interval times using the Wilcoxon test for the ITT and PP population.

8.2.8 TCD

The result of the TCD central assessment (Dr. Ritter, Münster) is a key element for the efficacy analysis of the study, see 8.4

The technical possibility to perform TCD (sufficient bone window) determines whether a patient is eligible for the study.

- TCD assessment was successful

Due to high rate of no microembolic signals we abandoned the criterion that if no microembolic signal was detected during the recording period then the patient was rated not eligible during the course of the study. In the further course of the study patients were included irrespective whether they present with microemboli or not.

8.2.9 DWI-NMR of eligible patients

Number of cerebral lesions in perfusion area of carotid artery stenosis is determined by a central reading lab (Dr. Hauser, Tübingen).

- back, front right and front left

Classes will be built for the number of lesions

0, 1, 2, 3-5, 6-10, >10

A homogeneity analysis will be performed for the number of cerebral lesions using the Mantel-Haenszel chi-square test for the ITT and PP population.

8.3 Duration of infusion

At Visit 2 study medication was administered by intravenous infusion for 20 minutes. As the start and end time of administration is documented the duration of infusion can be calculated.

- Duration (in minutes) of infusion will be summarized using descriptive statistics.

Comparability of groups will be assessed on base of duration of infusion using non-parametric Wilcoxon tests.

8.4 Efficacy analysis

All statistical tests will be performed two-sided and will be interpreted in a descriptive-exploratory way. Test for numerical data will be performed under normality assumptions using ANOVA. In addition normality of distribution will checked based on goodness of fit tests. If normality can not be assumed sensitivity analyses will be performed using the Wilcoxon test. Two-sided confidence intervals (CIs) will be displayed for important variables. Appropriate methods will be used to derive confidence intervals, depending on data nature and distribution.

8.4.1 Reduction of incidence of preoperative microembolic signals

This endpoint is to evaluate whether the rate of patients with incidence of preoperative microembolic signals (MES) was reduced (from Visit1 to Visit 3). Compared will be patients who have been treated with Revacept plus antiplatelet therapy (aspirin or clopidogrel) versus antiplatelet therapy alone (placebo) prior to carotid endarterectomy (CEA). MES data from the central reading lab (Dr. Ritter, Münster) are evaluated.

The evaluation will be performed using a two-sided test of superiority. Groups will be compared for the combined centers using Fisher's exact test.

The sequence of a priory defined hypotheses, with π rate of patients with MES V3 < MES V1 for the specified group, will be:

1) $H_{01}: \pi_{\text{Revacept 120 mg}} = \pi_{\text{Placebo}}$ vs.
   $H_{A1}: \pi_{\text{Revacept 120 mg}} > \pi_{\text{Placebo}}$

2) $H_{02}: \pi_{\text{Revacept 40 mg}} = \pi_{\text{Placebo}}$ vs.
   $H_{A2}: \pi_{\text{Revacept 40 mg}} > \pi_{\text{Placebo}}$

3) $H_{03}: \pi_{\text{Revacept 120 mg}} = \pi_{\text{Revacept 40 mg}}$
   $H_{A3}: \pi_{\text{Revacept 120 mg}} > \pi_{\text{Revacept 40 mg}}$

4) $H_{04}: \pi_{\text{Revacept 120 mg + 40 mg}} = \pi_{\text{Placebo}}$ vs.
   $H_{A4}: \pi_{\text{Revacept 120 mg + 40 mg}} > \pi_{\text{Placebo}}$

Each hypothesis will be tested using Fisher's exact test.

With the first two and the last of hypotheses the test product for a given dose level ot overall will be tested against Placebo. With the third hypothesis the higher dose level of the test product will be compared against the lower dose of the test product.

All tests will be interpreted exploratively.

The between group risk difference and its 95% confidence interval (CI) will be estimated for each group comparison.

The prime analysis will be based on the ITT population. The PP population will be used for the supportive analyses.
In a subgroup analysis, only patients will be compared who did not receive any change in the concomitant medication with anti-platelet activity (=rescue medication) between the initial assessment at V1 and the follow-up investigation at V3 / V5.

Additionally the prevalence / rate of patients with microembolic signals (>0) at Visit 3 will be compared in a similar manner as in the hypothesis 1- 4

Missing values for the primary analyses at Visit 3 will be imputed by the last observation carried forward approach. This means patients who dropped out before Visit 3 will be counted as non-responder.

An additional analysis of the first endpoint will be performed including the factor center as a stratum. Fisher's exact test will be performed for each stratum and meta-analysis approach will be chosen. Centers with less than 3 patients will be combined into a 'small number of patients' combined stratum.

Individual center results will be presented in a graphical overview with risk differences and their confidence intervals.

In case of heterogeneities in the baseline parameters, sensitivity analyses will be performed for the first endpoint using Fisher's exact test and by including the respective parameter as a stratum (binary, ordinal or categorical data). For continuous data these data will be transformed into classes before used as stratum.

A meta-analysis approach will be chosen to pool the stratum dependent results and to investigate for heterogeneity in the strata (e.g. Whitehead, 2003).

The following baseline parameters will be used for the analysis of heterogeneity:

- Demographics
  - Age
  - Height
  - Weight
  - Ethnic origin
  - Gender
  - Hormone status
- Anamnesis – Medical history
  - Number of patients with previous diseases and surgeries

- Vital signs
  - systolic and diastolic blood pressure (right and left arm)

- Physical examination
  - Abnormal findings of assessment of heart, lung, abdomen and oedema (Yes/No)

8.4.2 Rate of MES per hour (before and after treatment, before CEA)

The rate of MicroEmbolic Signals (MES) as assessed at Visit 1and at Visit 3 will be summarized using descriptive statistics. Data will be analysed from the central reading lab.

Groups will be compared for

- difference of MES at Visit 1 versus post treatment at Visit 3 (delta MES) and
- percent change of MES at Visit 3 compared to Visit 1 (delta MES)

using the ANOVA.

In a subgroup analysis, only patients will be compared who did not receive any change in the concomitant medication with anti-platelet activity (=rescue medication) between the initial assessment at V1 and the follow-up investigation at V3 / V5.

In addition normality of distribution will checked based on goodness of fit tests. If normality can not be assumed sensitivity analysis will be performed using the Wilcoxon test.

8.4.3 Assessment of neurological status (NIH Stroke Scale)

The neurological status was assessed at Visit 2, Visit 5, and Visit 6 by the NIH stroke scale. It comprises 15 items.

1a	Level of Conciousness		0	1	2	3		
1b	LOC Questions		0	1	2			
1c	LOC Commands		0	1	2			
2	Best Gaze		0	1	2			
3	Visual		0	1	2	3		
4	Facial Palsy		0	1	2	3		
5a	Motor Arm	left arm	0	1	2	3	4	9
5b		right arm	0	1	2	3	4	9
6a	Motor Leg	left leg	0	1	2	3	4	9
6b		right arm	0	1	2	3	4	9
7	Limb Ataxia		0	1	2			
8	Sensory		0	1	2			
9	Best Language		0	1	2	3		
10	Dysarthria		0	1	2			
11	Extinction and Inattention		0	1	2			
	Total Score		Maximum = 62					

with rating options on the scale 0, 1, 2, 3, 4 and 9 depending on the single item.

Frequencies of scale values will be summarized by visit and by item. Comparison between groups will be performed using the Mantel-Haenszel Chi-square test.

The total score of the NIH Stroke Scale will be compared between groups by visit using ANOVA.

8.4.4 Cerebral lesion analysis by DWI-NMR 1 day after CEA and correlation to neurological status

Cerebral lesions: Number of cerebral lesions in perfusion area of carotid artery stenosis assessed at Visit 5 by the central reading lab (Dr. Hauser, Tübingen).

- back
- front right
- front left
- and total

Groups will be compared for

- change of number of DWI lesions V5 compared to V1 (delta number of lesions)
- percent change of number of DWI lesions V5 compared to V1

using ANOVA.

In addition groups will be compared for

- rate of incidence of patients with DWI lesions after treatment (V5)
- rate of incidence of patients with new DWI lesions after treatment (V5)
- rate of patients with DWI lesions in V5 reduced compared to V1

using Fisher's exact test.

Neurological status: Modified Rankin Scale (MRS) assessed at Visit 6:

0	No symptoms at all
1	No significant disability despite symptoms; able to carry out all usual duties and activities
2	Slight disability; unable to carry out all previous activities, but able to look after own affairs without assistance
3	Moderate disability; requiring some help, but able to walk without assistance
4	Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability; bedridden, incontinent and requiring constant nursing care and attention
6	Dead

The following correlations will be performed by treatment group:

- Cerebral lesions of back area of carotid artery stenosis x MRS
- Cerebral lesions of front right area of carotid artery stenosis x MRS

- Cerebral lesions of front left area of carotid artery stenosis x MRS

Correlation analysis will be performed by calculating the Pearson correlation coefficient.
In a subgroup analysis, only patients will be compared who did not receive any change in the concomitant medication with anti-platelet activity (=rescue medication) between the initial assessment at V1 and the follow-up investigation at V3 / V5.

8.4.5 Clinical endpoints summarised cumulatively i.e. before treatment, before CEA, 1 day after CEA, at 3 months and at 12 months.

For the following endpoints

- Rate of all cause death
- Rate of stroke-related death
- Any TIA or stroke including haemorrhagic stroke
- Rate of Myocardial infarctions and coronary re-intervention

the number of patients will be summarized by means of frequency tables. Groups will be compared using Fisher's exact test.

8.4.6 Ischemic events and rate of rescue medication during the study

Combined clinical endpoints for ischemic events (myocardial infarction and ischemic stroke/TIA) and rate of rescue medication (additional anti-platelet co-medication) during the study will be analyzed comparing groups for

- incidence of events adjusted for application of rescue medication during the study (yes/no)

using the Cochran-Mantel-Haenszel procedure.

8.5 Assessment of safety

Safety outcome between groups and between placebo and the combined Revacept groups will be compared.

8.5.1 Vital signs

Vital signs were assessed at Screening (Visit 1, Baseline) and Visit 2 to Visit 6.

Results of

- vital signs
  - Weight
  - Blood pressure systolic and diastolic (right arm / left arm)

will be summarized by visit by means of descriptive statistics. Change from baseline in weight, heart rate, systolic and diastolic blood pressure will be summarized by means of statistics and compared between treatment groups using the Wilcoxon test.

8.5.2 Physical examination

Physical examination was assessed at Screening (Visit 1, Baseline) and Visit 2 to Visit 6.

Results of assessment of

- heart
- lung
- abdomen
- oedema

will be described presenting number and percent of patients with abnormal findings at post-baseline visits and with normal values at baseline (Screening, Visit 1) and summarized by means of frequency tables.

8.5.3 ECG parameters

ECG parameters which were assessed at Screening (Visit 1, Baseline), Visit 3, Visit 5 and Visit 6 are heart rate, type of rhythm, clinically significant ST-segment deviation and interval times.

Results for

- Heart rate [beats/minute]

will be summarized by means of descriptive statistics

- Type of rhythm
  - Sinus rhythms
  - Atrial fibrilation
  - Other rhythm

will be summarized by means of frequency tables

- Clinically significant ST-segment deviation

will be summarized by means of frequency tables

- Interval times

- P [ms]
- PQ interval [ms]
- QRS complex [ms]
- QTc [ms]

will be summarized by means of descriptive statistics

Change from baseline in heart rate, and interval times will be summarized by means of statistics and compared between treatment groups.

8.5.4 Adverse events

Adverse events will be categorised by primary system organ class (SOC) and MedDRA preferred term (PT) as coded using the MedDRA dictionary.

- The inquiries for AEs will be summarised with respect to the type of inquiry
  - Patient showed up for follow up visit and AE/SAE was reported during a follow up visit
  - Patient did not show up for follow-up visit and AE/SAE was reported by telephone or in writing with
    - the patient
    - a family member
    - an attending physician
    - another person

- An overview table will be presented with the number (and percentage) of patients with at least one AE, with at least one SAE, with AEs leading to treatment discontinuation and with drug-related AEs.
- The number (%) of patients with at least one AE will be presented in frequency tables by adverse events as reported.

8.5.5 Laboratory results

The following endpoints will be evaluated exploratory using descriptive methods of statistics. Generated p-values will be interpreted in an explorative way.

- Laboratory results
  - Biochemistry
  - Haematology
  - Coagulation
  - Urinanalysis

- Bleeding

- Reduction in haemoglobin level
  - by at least 20 g/L
  - by at least 50 g/L
  - of ≥ 2 units of blood
  - of ≥ 4 units of blood
- Requiring transfusion
  - of ≥2 units of blood
  - of ≥4 units of blood
- Symptomatic bleeding in a critical area of organ
  - Intra-ocular
  - Intra-spiral
  - Intra-musculär
  - Retro-peritoneal
  - Intra-articular
  - Pericardial
  - Subdural intra-cranial
  - Intra-cerebral
- Other symptomatic bleeding
  - Gastrointestinal
  - Other organ
- Involving hypotension
- Surgery necessary
- Fatal

8.5.6 Pharmacokinetics

Analyses of anti drug antibodies and Revacept pharmacokinetics will be conducted at the AdvanceCor GmbH according to validated protocols and SOPs effective at that moment in time.

8.6 Change in concomitant medication

- Change in concomitant medication will be described presenting
  - number and percent of patients with new concomitant medication - taken first time after start of treatment (during the course of the study)
  - number and percent of patients with changes of pre-existing concomitant medication according to
    - dose increased
    - dose decreased
    - discontinued
  - type of medication

- type of medication by indication

and summarized by means of frequency tables.

- Change in concomitant rescue medication with anti-platelet or anti-thrombotic effects.

8.7 Individual patient listing

Individual patient listings will be produced for all raw data and a selection of the derived data. Listings will be ordered by treatment group, and by gender and study centre within treatment group.

9. LITERATURE

1. Whitehead, Anne (2003) Meta-Analysis of Controlled Clinical Trials. Statistics in Practice