



Ouabain and Adducin for Specific Intervention on Sodium in HyperTension (OASIS-HT): design of a pharmacogenomic dose-finding study

Jan A Staessen¹, Tatiana Kuznetsova¹, Rok Acceto², Antonella Bacchieri³, Eva Brand⁴, Michel Burnier⁵, Hilde Celis¹, Lorena Citterio⁶, Peter W de Leeuw⁷, Jan Filipovsky⁸, Albert Fournier⁹, Kalina Kawecka-Jaszcz¹⁰, Paolo Manunta⁶, Yuri Nikitin¹¹, Eoin T O'Brien¹², Josep Redón¹³, Lutgarde Thijs¹, Patrizia Ferrari¹⁴, Giovanni Valentini³, and Giuseppe Bianchi⁶, on behalf of the OASIS-HT Investigators

¹Study Coordinating Centre, Hypertension and Cardiovascular Rehabilitation Unit, Department of Molecular and Cardiovascular Research, University of Leuven, Belgium; ²Hypertension Department, University Medical Centre, Ljubljana, Slovenia; ³Sigma Tau Industrie Farmaceutiche Reunite, Pomezia, Italy; ⁴Department of Internal Medicine D, University Hospital of Münster, Germany; ⁵Division of Hypertension and Cardiovascular Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland; ⁶Divisione di Nefrologia Dialisi e Ipertensione, Ospedale San Raffaele, Dipartimento di Scienze e Tecnologia Biomediche, Università Vita-Salute, Milan, Italy; ⁷Department of Medicine, University Hospital of Maastricht, The Netherlands; ⁸Department of Internal Medicine 2, Charles University, Pilsen, The Czech Republic; ⁹Department of Internal Medicine, Centre Hospitalier Universitaire Amiens, France; ¹⁰First Cardiac Department, Jagiellonian University, Krakow, Poland; ¹¹Institute of Internal Medicine, Novosibirsk, Russian Federation; ¹²ADAPT Centre and Blood Pressure Unit, Beaumont Hospital and Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin, Ireland; ¹³Hospital Clínico Universitario Valencia, University of Valencia, Spain; ¹⁴Praxis Research Institute, Settimo Milanese, Italy

Contents

1.	Introduction	758
2.	Characteristics of rosfuroxin	759
2.1	<i>Pharmacologic properties</i>	759
2.2	<i>Toxicology</i>	759
2.3	<i>Phase I studies</i>	760
2.4	<i>Phase II studies</i>	760
3.	Design of the OASIS-HT trial	760
3.1	<i>Objectives</i>	760
3.2	<i>Experimental design</i>	760
3.3	<i>Selection of patients</i>	761
3.3.1	Positive selection criteria	761
3.3.2	Exclusion criteria	762
3.4	<i>Randomization</i>	762
3.4	<i>Overview of the study procedure</i>	762
4.	Treatment of patients	762
4.1	<i>Lifestyle</i>	762
4.2	<i>Study medication</i>	763
4.3	<i>Use of other medications</i>	763
5.	Measurement of phenotypes	763
5.1	<i>Guidelines for blood-pressure measurements</i>	763
5.1.1	Selection of the cuff size	763
5.1.2	Selection of the arm	763
5.1.3	Logging of devices	764
5.2	<i>Clinic blood pressure</i>	764
5.3	<i>Ambulatory blood-pressure monitoring</i>	764
5.3.1	Selection of the ambulatory blood pressure monitoring device	764
5.3.2	Instructions for investigators	764
5.3.3	Instructions for patients	765
5.3.4	Quality assurance	765
5.4	<i>Biochemical phenotypes</i>	765
5.5	<i>The 24-h urine collection</i>	765

5.6	<i>Electrocardiography</i>	766
5.6.1	Registration of electrocardiograms	766
5.6.2	Phenotypes to be determined from the electrocardiogram	767
6.	Measurement of genotypes	767
7.	Discontinuation of randomized treatment	767
7.1	<i>Withdrawals</i>	768
7.2	<i>Defectors</i>	768
7.3	<i>Definition and documentation of adverse events</i>	768
7.3.1	Serious adverse events	768
7.3.2	Nonserious adverse events	768
8.	Statistical methods	768
8.1	<i>Sample size</i>	768
8.2	<i>Patient populations to be analyzed</i>	768
8.3	<i>Analysis of primary and secondary end points</i>	769
8.4	<i>Analysis of the ambulatory recordings</i>	769
8.5	<i>Analysis of safety</i>	769
9.	Ethics	770
10.	Discussion	770
11.	Conclusion	772
	Acknowledgments	772
	Bibliography	772
	Appendix	775



OASIS-HT: design of a pharmacogenomic dose-finding study

Jan A Staessen,
Tatiana Kuznetsova,
Rok Acceto,
Antonella Bacchieri,
Eva Brand,
Michel Burnier,
Hilde Celis,
Lorena Citterio,
Peter W de Leeuw,
Jan Filipovský,
Albert Fournier,
Kalina Kawecka-Jaszcz,
Paolo Manunta,
Yuri Nikitin,
Eoin T O'Brien,
Josep Redón,
Lutgarde Thijs,
Patrizia Ferrari,
Giovanni Valentini &
Giuseppe Bianchi

Experimental evidence and observations in humans strongly support an interactive role of mutated α -adducin, sodium (Na^+)/potassium (K^+)-adenosine triphosphatase (ATPase) activity and endogenous ouabain in Na^+ homeostasis and the pathogenesis of hypertension. The Ouabain and Adducin for Specific Intervention on Sodium in HyperTension (OASIS-HT) trial is an early Phase II dose-finding study, which will be conducted across 39 European centers. Following a run-in period of 4 weeks without treatment, eligible patients will be randomized to one of five oral doses of rostaduroxin consisting of 0.05, 0.15, 0.5, 1.5, or 5.0 mg/day. Each dose will be compared to a placebo in a double-blind crossover experiment with balanced randomization. Treatment will be initiated with the active drug and continued with placebo or *vice versa*. Each double-blind period will last 5 weeks. The primary end point is the reduction in systolic blood pressure defined as the average of three clinic readings with the patient in the sitting position. Secondary end points include the reduction in diastolic blood pressure on clinic measurement, the decrease in the 24-h blood pressure, and the incidence of end points related to safety. Secondary objectives are to investigate the dependence of the blood pressure-lowering activity on the plasma concentration of endogenous ouabain and the genetic variation of the enzymes involved in the metabolism of this hormone, and the adducin cytoskeleton proteins. Eligible patients will have Grade I or II systolic hypertension without associated conditions and no more than two additional risk factors. In conclusion, OASIS-HT is a combination of five concurrent crossover studies, one for each dose of rostaduroxin to be studied. To our knowledge, OASIS-HT is the first Phase II dose-finding study in which a genetic hypothesis is driving primary and secondary end points.

1. Introduction

Adducin is a heterodimeric cytoskeleton protein consisting either of α - and β -subunits or of α - and γ -subunits [1,2]. Rat experiments [1,3], *in vitro* transfection studies [4], sodium-loading and -depleting interventions in never-treated hypertensive patients [5] and, finally, epidemiologic studies [6,7] revealed a sequence of events leading from a point mutation in the α -subunit [3] to a cellular dysfunction characterized by higher activity of the sodium pump [4]. This leads to increased tubular sodium reabsorption in the kidney [5] and, ultimately, hypertension [6].

Endogenous ouabain is released from the mid-brain and possibly from the adrenal glands [8] in response to hypoxia, expansion of the extracellular fluid volume induced by aldosterone, or in response to a low-salt diet [9]. The hypothesis gaining ground is that, depending on its concentration and the experimental conditions, this hormone behaves as a versatile modulator of the ubiquitously expressed sodium pump [10]. At high concentrations, ouabain inhibits the

sodium (Na^+)/potassium (K^+)-adenosine triphosphatase (ATPase) activity in renal tubular cells, promotes natriuresis and reduces blood pressure. In cardiac and vascular myocytes, inhibition of Na^+/K^+ -ATPase activity may slow the sarcolemmal Na^+ -calcium (Ca^{2+}) exchange and, via Ca^{2+} -dependent pathways, stimulate excitation-contraction coupling, leading to vasoconstriction and the expression of growth-related genes [11,12]. At very low concentrations within the subnanomolar range, endogenous ouabain may increase the size of the membrane pool of active Na^+ pumps [13] and, via this or other unknown mechanisms, lead to renal Na^+ conservation rather than salt losing [10], hypertension, and hypertrophy of target organs, such as the heart or the kidney.

Milan hypertensive rats strains (MHS) carry a mutated α -adducin and, compared with the normotensive control strain (MNS), have higher circulating levels of endogenous ouabain. Introgression of the DNA segment containing the mutated adducin locus from MHS into

Keywords: adducin, dose-finding, hypertension, ouabain, pharmacogenomic, Phase II, rostaduroxin, sodium, trial

future
medicine

MNS increases blood pressure and, under Na^+ -deplete conditions, also raises the plasma concentration of ouabain. Furthermore, in subjects randomly recruited from a Caucasian population, whose plasma ouabain concentration was less than 140 pmol/l (median), blood pressure increased by 2.2 mmHg systolic and 1.4 mmHg diastolic for each 50 mmol increment in their 24-hour urinary Na^+ excretion [14]. In contrast, no association between blood pressure and the urinary Na^+ excretion was found when plasma ouabain exceeded the median [14]. Moreover, both before and after adjustment for sex and smoking, plasma ouabain significantly increased with the number of copies of the mutated α -adducin *460Trp* allele. Thus, observations in rats and humans strongly support an interactive role of mutated α -adducin, Na^+/K^+ -ATPase activity, and endogenous ouabain in the regulation of Na^+ homeostasis and the pathogenesis of hypertension.

2. Characteristics of rostafuroxin

Rostafuroxin (17 β -[3-furyl]-5 β -androstan-3 β ,14 β ,17 α -triol) is a digitoxigenin derivative (Figure 1), which selectively displaces ouabain from the Na^+/K^+ -ATPase receptor [15].

2.1 Pharmacologic properties

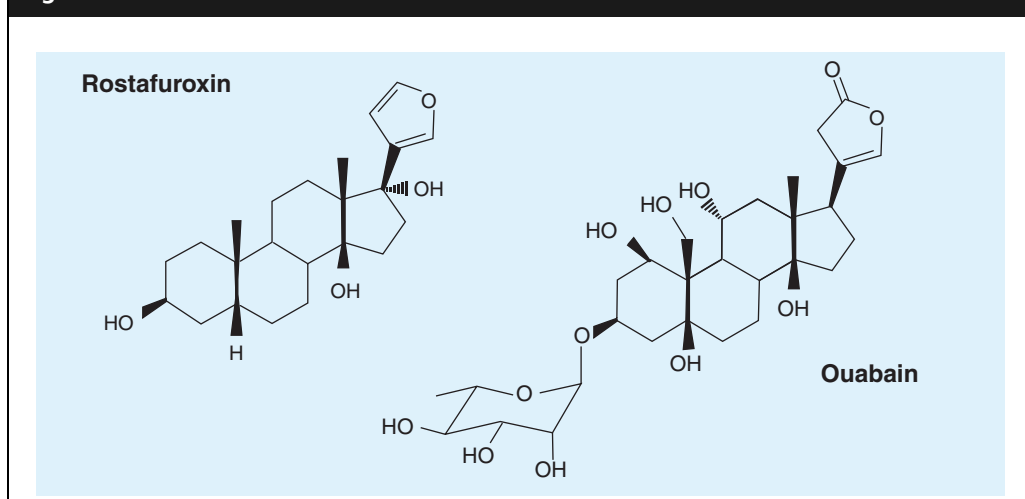
In MHS rats, at doses of 100 $\mu\text{g}/\text{kg}$ or higher, rostafuroxin inhibited the innate excess activity of the Na^+ pump [16] and approximately halved the differences in the blood pressure levels compared with normotensive MNS controls. In MNS made hypertensive by the chronic infusion of ouabain at doses from 15–50 $\mu\text{g}/\text{kg}/\text{day}$, rostafuroxin administered in daily doses of 0.1–10 $\mu\text{g}/\text{kg}$ also normalized the increased renal Na^+/K^+ -ATPase activity and blood pressure, and antagonized the hypertrophy of the heart and kidneys. This antihypertropic effect may not only be related to the blood pressure-lowering effect of rostafuroxin, as a similar blood pressure decrease produced by the dihydropyridine calcium channel blocker amlodipine did not reduce cardiac and renal hypertrophy. Rostafuroxin inhibited the interaction between ouabain and the Na^+ pump, which, *in vitro*, activates a mitogen-activated protein kinase-related pathway leading to cell growth [17]. In isolated tubular cells, rostafuroxin at concentrations of 10^{-9} or 10^{-10} M normalized the increase in the Na^+/K^+ -ATPase activity produced either by transfection of these cells by mutated MHS adducin, or by their exposure to subnanomolar concentrations of ouabain (10^{-11} or 10^{-12} M). In

experimental animals and cell models, rostafuroxin inhibited the ouabain-mediated alterations at doses or concentrations that were approximately tenfold lower than those required for antagonizing the effects of mutated α -adducin [18]. Up to concentrations of 10^{-4} M, rostafuroxin does not interact with receptors involved in the regulation of blood pressure or endocrine function. Rostafuroxin does not behave as a diuretic, does not decrease the creatinine clearance, does not activate the renin–angiotensin–aldosterone system, and does not induce the metabolic side effects of diuretics, such as potassium depletion, insulin resistance or dyslipidemia [16,18].

Pharmacokinetic studies showed that rostafuroxin is orally absorbed and subsequently metabolized. The compound is bound to plasma proteins in 90% of cases. It has a 90% hepatic extraction with negligible renal clearance. In rats, monkeys and humans, the 3 α -epimer of the parent compound (PST2490) is a metabolite that can be detected in urine. This observation suggests a common metabolic pathway in the three species. After intravenous or oral administration, the estimated half-life of the unchanged drug is approximately 12 min or 3.5 h, respectively. The bioavailability computed from the area under the curve after intravenous or oral administration with standardization for dose is approximately 2.1%. The relatively short half-life of the unchanged compound, the long lasting antihypertensive effect that, following oral administration, persists for more than 24 h, and the fact that the metabolite PST 2490 is devoid of blood pressure lowering activity suggest compartmentalization in target organs, from which the compound may be slowly released.

2.2 Toxicology

The acute oral lethal dose resulting in 50% mortality (LD_{50}) in rats and mice exceeds 2000 mg/kg. In 1-month oral toxicity studies in rats, a dose of 45–180 mg/kg caused mild hematologic and hepatic alterations, which were not dose dependent. A dose of 720 mg/kg was lethal for 60% of male rats, but not for female rats. Similar 1-month studies in monkeys with the same oral doses of rostafuroxin revealed slight alterations in the hematologic and hepatic measurements, but did not cause any death, not even at the highest dose. At the lowest dose, monkeys did not experience any toxic effect. Chronic oral toxicity studies over 3 months did not reveal any toxicity up to a dose of 100 mg/kg in rats and 180 mg/kg in monkeys.

Figure 1. Chemical structure of rostafuroxin and ouabain.

Rostafuroxin was devoid of mutagenicity in human lymphocytes, Chinese hamster lung cells and the bone marrow of mice. At a dose of 400 mg/kg, rostafuroxin decreased spermatogenesis in male rats, and in female rats and rabbits followed up until the 7th day of pregnancy it led to disturbances in the embryo–fetal development.

2.3 Phase I studies

In placebo-controlled studies in healthy male volunteers who received rostafuroxin in a daily dose ranging from 1–10 mg for up to 7 days, no clinically significant adverse effects occurred. In particular, the compound had no influence on electrocardiographic measurements, including the RR, PR, QRS, and QS intervals. Following the initial dose, a few volunteers in the active-treatment and placebo groups had mild complaints, mainly headaches, which all subsided without sequels. After glucuronide hydrolysis, the parent compound was undetectable in urine, but the excretion of the urinary metabolite (PST2490) increased linearly with the orally administered dose.

2.4 Phase II studies

In an uncontrolled pilot study with forced titration, never-treated hypertensive patients received rostafuroxin during three sequential periods of 1 month in daily doses of 0.1, 1 and 5 mg, respectively. Compared with the baseline, the decreases in mean arterial pressure averaged 3.0 mmHg ($p = 0.039$), 5.0 mmHg ($p = 0.007$), and 5.0 mmHg ($p = 0.014$), respectively.

In a second study with double-blind design, 42 and 21 patients with uncomplicated hypertension were randomized to 12 weeks of treatment with

either rostafuroxin 0.5 mg/day or losartan 50 mg/day. The intention-to-treat analysis showed that at the end of follow up blood pressure was slightly higher with rostafuroxin than losartan, but that the proportion of patients whose diastolic blood pressure dropped to 90 mmHg or less was 38.0% in the two groups. Mild adverse events were equally frequent (19.0%) on both drugs and, with the exception of one case of gastritis on rostafuroxin, they were all considered to be unrelated to treatment. No serious adverse events occurred.

3. Design of the OASIS-HT trial

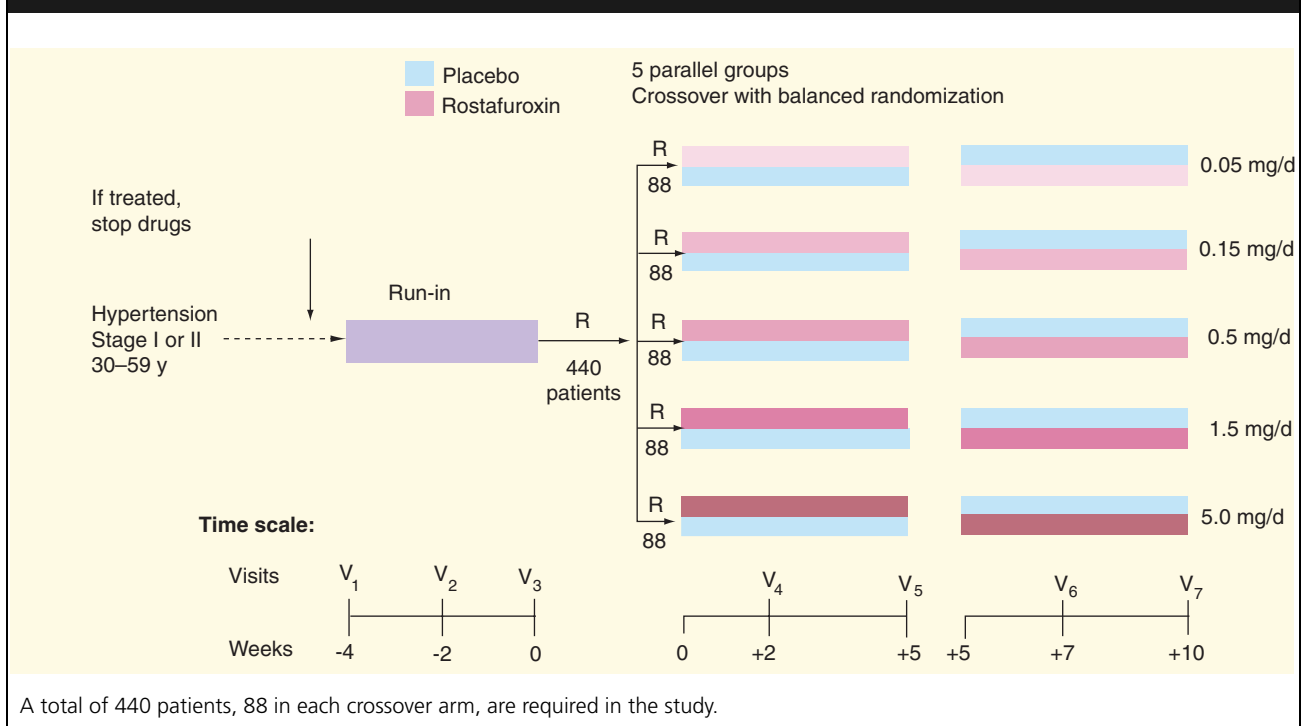
3.1 Objectives

The primary objective of the double-blind OASIS-HT trial is to identify the minimal daily dose at which rostafuroxin in patients with uncomplicated hypertension reduces blood pressure significantly more than placebo. The primary end point is the reduction in systolic blood pressure defined as the average of three clinic readings with the patient in the sitting position. Secondary end points include the reduction in diastolic blood pressure on clinic measurement, the decrease in the 24-h blood pressure, and the incidence of end points related to safety. An important secondary objective is to investigate the dependence of the blood pressure lowering activity on the plasma concentration of endogenous ouabain, and on the genetic variation in the enzymes involved in the synthesis of this hormone and the adducin cytoskeleton proteins.

3.2 Experimental design

OASIS-HT is an early Phase II dose-finding study, which will be conducted across 39 European

Figure 2. Diagrammatic representation of the study protocol.



centers. After a run-in period of 4 weeks, eligible patients will be randomized to one of five oral doses of rostafuroxin consisting of 0.05, 0.15, 0.5, 1.5, or 5.0 mg/day. Each dose will be compared with placebo in a double-blind crossover experiment with balanced randomization. Treatment will be initiated with the active drug and continued with a placebo or *vice versa*. Each double-blind period lasts 5 weeks. OASIS-HT is therefore a combination of five concurrent crossover studies, one for each dose of rostafuroxin to be studied. The total duration of the study, including the run-in period, is 14 weeks. The wide dose range used in OASIS-HT is based on the preclinical studies that showed rostafuroxin inhibited the ouabain-mediated changes at doses or concentrations that were approximately tenfold lower than those required for antagonizing the effects of mutated α -adducin [16,18].

3.3 Selection of patients

Patients with hypertension qualify for randomization if they meet all entry criteria and if they have none of the characteristics leading to exclusion.

3.3.1 Positive selection criteria

Female and male patients are eligible in the presence of additional positive selection criteria:

- Age should range from 30–59 years.
- At the screening visit, patients should be

untreated or on treatment with only one drug or one combination tablet containing no more than two antihypertensive agents. It should be possible to stop antihypertensive drug treatment at the screening visit.

- Patients should have Grade I or II hypertension according to the 2003 European guidelines [19] without any associated conditions. At the screening visit, systolic blood pressure must range from 140–169 mmHg, irrespective of treatment status. Two weeks later, while the patients are untreated, their systolic blood pressure must remain higher than 140 mmHg. At the end of the run-in period, 4 weeks after the screening visit, the untreated systolic blood pressure must range from 140–169 mmHg.
- In addition to hypertension, patients should have less than three other risk factors including: age above 55 years if male, smoking, total cholesterol of 250 mg/dl or higher, low-density-lipoprotein cholesterol of 155 mg/dl or higher, or family history of cardiovascular disease occurring before 55 years in men or 65 years in women.
- Women with childbearing potential should apply an adequate contraceptive technique.
- Patients should provide written informed consent.

3.3.2 Exclusion criteria

The following criteria lead to exclusion:

- A 24-h urinary Na⁺ excretion exceeding 200 mmol at the last run-in visit.
- Severe or malignant hypertension or secondary hypertension (which includes a history of renal arterial disease).
- Disturbances of cardiac rhythm or conduction, including atrial fibrillation, first degree atrioventricular block with a PR-interval of 240 ms or longer, or left or right ventricle bundle branch block.
- Electrocardiographic evidence of left ventricular hypertrophy [20–22], defined as a Sokolow-Lyon index of 38 mm or more or as a Cornell voltage product exceeding 2440 mm × ms.
- A history of myocardial infarction within 6 months of randomization or heart disease requiring the intake of blood pressure lowering medications, vasodilators, antiarrhythmic drugs, or digitalis.
- Diseases of the kidney or liver as evidenced by a serum creatinine concentration of 1.3 mg/dl or higher, microalbuminuria in excess of 2.5 mg per mmol of creatinine in men or 3.5 mg per mmol of creatinine in women, or a twofold or higher increase in alanine or aspartate aminotransferase activity in serum compared with the normal reference values.
- Surgery or diseases of the gastrointestinal system, which might influence the absorption or hepatic clearance of rofuroxin.
- Pregnant or nursing women, or women with childbearing potential not applying adequate contraception.
- Metabolic disturbances, including a body mass index exceeding 30 kg/m² or overt or medically-treated diabetes mellitus.
- Any condition that might be predictive of a lack of collaboration, such as substance abuse or mental disorders.
- Treatment with any other investigational drug within 6 months of randomization.

3.4 Randomization

Randomization will follow a balanced incomplete block design. Each center will be randomly allocated to one or more incomplete blocks. Within blocks, patients will be randomly assigned to four or six of the ten possible sequences of rofuroxin (five doses) and placebo (either preceding or following the

active drug). A computerized procedure generating random numbers will be used for randomization.

Centers will opt whether they will use blocks of study medication for four or six patients. A copy of the randomization code of each individual patient will be kept in sealed envelopes at the Drug Safety Unit (Sigma-Tau, Pomezia, Italy) and at each center. Breaking of the code is only allowed when, in the patient's best interests, a serious adverse event cannot be handled otherwise. Code breaks should be recorded in the case report forms.

3.5 Overview of the study procedures

At the initial screening visit (V1, -4 weeks), investigators will assess the potential eligibility of patients, discontinue antihypertensive drug treatment, obtain informed written consent, and plan further follow-up during the run-in period, while the patients remain off antihypertensive drug treatment. Eligibility will be further assessed at visit 2 (V2, -2 weeks) and visit 3 (V3, baseline).

Patients complying with all enrolment criteria will be randomized to the double-blind study medication. They will be started on active study medication for 5 weeks and then be crossed over to placebo, or *vice versa*. Each of these two treatment periods runs over 5 weeks. Short visits (V4 and V6) are scheduled 2 weeks after the initiation of each course of double-blind medication and visits with an extensive evaluation are scheduled at the end of each treatment period (V5 and V8). The time window allowed for each visit is the programmed time plus up to 7 days. Figure 3 provides an overview of the examinations to be performed at each visit.

4. Treatment of patients

The 440 patients randomized in OASIS-HT will be treated according to the 2003 guidelines of the European Society of Hypertension and the European Society of Cardiology [19].

4.1 Lifestyle

Patients eligible for enrolment in OASIS-HT have mild hypertension, Grade I or II, without associated conditions and no more than two additional cardiovascular risk factors. In keeping with current guidelines [19], the treatment strategy in such patients should, for several months, consist of the reinforcement of lifestyle measures and regular follow-up. The hygienic measures to be implemented include cessation of smoking,

moderation of excessive alcohol intake, regular physical activity, weight reduction in overweight patients, dietary measures to control dyslipidemia, and, most importantly, in relation to the hypothesis under investigation a reduction of dietary Na⁺ intake to between 120 and 160 mmol/day.

4.2 Study medication

The pharmacist of each center will be responsible for the receipt and storage of the study medication at a secure location and at a room temperature below 25 °C. The study medication will be provided as gelatinized capsules containing 0.05, 0.15, 0.5, 1.5, or 5.0 mg of the active substance and matching placebo capsules. For each patient, the parcel will include two boxes, labeled ‘period 1’ or ‘period 2’. Each box will contain two bottles, respectively labeled ‘weeks 1–2’ or ‘weeks 3–5’ with sufficient capsules (21 or 28, respectively) for the 2- or 3-week treatment periods plus 7 extra days. All labels will be printed in the language of the center. The label of each bottle has a tear-off part, which fully identifies the bottle and the patient and which the investigator has to stick onto the case record form when the study medication is dispensed.

Patients should take the study medication around 8 AM before breakfast. Clinic visits have to be scheduled within 8 h of drug intake. Depending on local conditions, each center should standardize the time interval between the intake of the study medication and the measurement of the clinic blood pressure. On the days before visits five and seven, the patients should go to the clinic following overnight fasting. On these days, they should take their study medication at the examination center after blood sampling and shortly before the start of the ambulatory blood pressure recording and the 24-h urine collection.

Patients should return unused capsules at the next visit. The investigator will note the number of unused capsules on the case record forms and return unused or partially used bottles of study medication to the sponsor at the end of the trial. Patients will be classified as compliant if they took at least 80% of the prescribed study medication and if they did not miss any dose on the days of the clinic visits and the days immediately preceding these visits.

4.3 Use of other medications

Patients cannot use medications that might have an influence on the outcome variables under study:

- Drugs used for the treatment of cardiovascular disorders, including diuretics, α - and β -blockers, calcium-channel blockers, angiotensin-converting enzyme inhibitors, angiotensin II type-1 receptor blockers, and centrally acting antihypertensive drugs.
- Medications causing systemic vasodilatation or vasoconstriction, including theophylline, papaverine, tricyclic antidepressants, neuroleptics, nitrates, and sympathicomimetic drugs in aerosol for bronchial or nasal application.
- Digitalis and antiarrhythmic agents.
- Steroids and nonsteroidal anti-inflammatory drugs, with the exception of aspirin, at a dose of less than 100 mg/day or at a higher dose for a period of less than 1 week.
- Immunosuppressive or cytotoxic agents.
- Potassium supplements.

5. Measurement of phenotypes

High-fidelity phenotyping is a cornerstone to the research goals of OASIS-HT.

5.1 Guidelines for blood-pressure measurements

The blood pressure measured at the clinic is the primary outcome variable in the OASIS-HT trial. Blood pressure will also be monitored under ambulatory conditions. Investigators will have to comply with the recommendations of the European Society of Hypertension for conventional and ambulatory blood-pressure measurement [23].

For measurement of blood pressure at the clinic, all centers will employ validated [24] oscillometric Omron 705IT recorders (Omron Healthcare Europe BV, Nieuwegein, The Netherlands). Although for ambulatory monitoring any validated ambulatory recorder can be employed, most centers will use oscillometric SpaceLabs 90207 devices [25]. The following guidelines for standardization apply equally to clinic blood pressure measurement and ambulatory monitoring.

5.1.1 Selection of the cuff size

At the screening visit, the investigator must determine the appropriate cuff size for each patient. If arm circumference is less than 32 cm, a standard cuff with a bladder of 12 × 22 cm can be applied. For arms with a larger circumference, cuffs must have a bladder size of at least 15 × 31 cm.

5.1.2 Selection of the arm

At the screening visit, the investigator must ascertain that there is no clinically significant difference in the brachial blood pressure as measured on the

left and right arm. After the patient has rested for 5 min in the sitting position, the observer must, within an interval of less than 1 min, obtain one blood-pressure reading on each arm, always starting with the nondominant arm. If the systolic and diastolic differences in blood pressure between both arms are less than 10 mmHg, all blood-pressure readings must be obtained on the nondominant arm. Otherwise, the arm giving the highest blood pressure readings must be chosen.

5.1.3 Logging of devices

A unique number will identify each electronic device used for blood pressure measurement at the clinic, or under ambulatory conditions. For each apparatus, investigators must maintain a log book documenting checks of calibration, defects and repairs, and other maintenance procedures, such as the purchase of a new cuff or bladder. Throughout the trial, each patient must be followed up using the same devices applied to the same arm by means of equally sized cuffs.

5.2 Clinic blood pressure

The investigator will measure the clinic blood pressure with the Omron 705IT device after the patient has been seated for 5 min in a quiet room, according to the 2003 European guidelines [23]. The patient's chair should provide comfortable back support. If possible, the same observer must measure each patient's clinic blood pressure, always in the same examination room and at approximately the same time of the day.

Investigators should be familiar with the manual of operations of the Omron 705IT monitor and proceed as outlined below:

- The investigator should first clear the memory.
- After the patient has rested for 5 min or longer, the observer will obtain five consecutive blood pressure readings at intervals of 30–60 s, while the patient remains in the sitting position. The investigator will then ask the patient to stand up. Immediately after the patient has assumed the upright position, the observer will obtain two additional blood pressure readings. The results of all the readings must be immediately recorded onto the study forms, as well as the value of the pulse rate at the last of the sitting and standing measurements. The last three measurements in the sitting position should be averaged and also recorded onto the patient form.
- Immediately after the five sitting, and two standing, blood-pressure measurements have

been recorded, the investigator must print these values (systolic and diastolic blood pressure, pulse rate and time of day), label the paper strip with the patient's randomization number, initials, center code, and date, and clip the paper strip to the patient form.

5.3 Ambulatory blood pressure monitoring

5.3.1 Selection of the ambulatory blood pressure monitoring device

The devices used in OASIS-HT must have passed validation according to the guidelines of the Association for the Automation of Medical Instrumentation (AAMI [26]), the British Hypertension Society [27], or the International Protocol [24]. The recorders should be programmed to obtain measurements every 15 min from 8 AM–10 PM and every 30 min for the remainder of the day. An updated list of validated devices is available at [101].

5.3.2 Instructions for investigators

The 24-h ambulatory blood pressure recordings should be initiated and downloaded from the monitor in a standardized way:

- Before the recording, the investigator must check whether the memory of the recorder has been cleared and whether the patient identification has been correctly entered.
- After the patient has been seated for at least 5 min, the cuff of the blood pressure monitor is applied to the arm and two test readings are obtained with the ambulatory monitor. The cuff of the monitor is then replaced by that of the Omron device. Two blood pressure readings are obtained by means of the Omron device and noted on the patient's diary card. Thereafter, the cuff of the ambulatory monitor is again applied to the arm and two further test readings are obtained by means of the ambulatory monitor.
- The patient is briefed on the technical details of the recording and is given the diary card, which has to be completed during the recording. The patient must also receive a telephone number, where a doctor or technician can be called should technical problems arise during the recording.
- Immediately after the recording, the investigator must print a hard copy of the results and clip it to the patient form. In addition, the investigator must also forward the recording and the patient's diary card to the Study Coordinating Center in Leuven, Belgium.

Editing of the ambulatory recordings is not allowed. Editing criteria encoded in the monitor must be disabled. If this feature cannot be programmed off, limits should be set as wide as possible.

5.3.3 Instructions for patients

The patients will be encouraged to wear the monitors for at least 25 h to ensure that blood pressure readings are collected over a full 24-h period. When awake, patients must hold their arm still during inflation of the cuff. On the days of the recordings, the patients will complete a diary card, from which it must be possible to retrieve when sleep at night commenced and ended. Sleep is defined as the period elapsing from the moment when the patient went to bed with the intention to sleep to the time of the first visual sensation after sleep. In addition, the diary card must report symptoms, if any, the time of their occurrence, the intake of medications, and indicate when during the day the main meal was consumed.

5.3.4 Quality assurance

The ambulatory recordings must comply with the following quality criteria:

- At least ten readings from 10 AM–10 PM and five from midnight–6 AM.
- The whole recording should cover at least 20 h with no interval between two successive readings exceeding 3 h.

5.4 Biochemical phenotypes

At visits three, five and seven (Figure 3), venous blood must be collected after overnight fasting and prior to intake of the study medication. Blood samples are required for:

- DNA extraction and amplification and genotyping (only at visit 3).
- Measurement related to Na⁺ homeostasis, including plasma renin activity and the serum concentrations of Na⁺, potassium, endogenous ouabain, and aldosterone.
- Measurements related to drug safety:
 - Hematological determinations, including hemoglobin, hematocrit, red blood cell count, total and differential counts of white blood cells, and platelet count.
 - The serum concentrations of calcium, inorganic phosphorus, total protein, albumin, total bilirubin, total cholesterol, triglycerides, uric acid, and creatinine.

- The activity in serum of enzymes, including alkaline phosphatase, alanine (ALT/GPT) and aspartate (AST/GOT) aminotransferase, and γ -glutamyl-aminotransferase.

At visits 3, 5 and 7 (Figure 3), the patients should also provide a fresh urine sample for measurement of specific gravity and pH, dipstick measurement of glucose, ketones, protein, hemoglobin and bilirubin, and a microscopic examination.

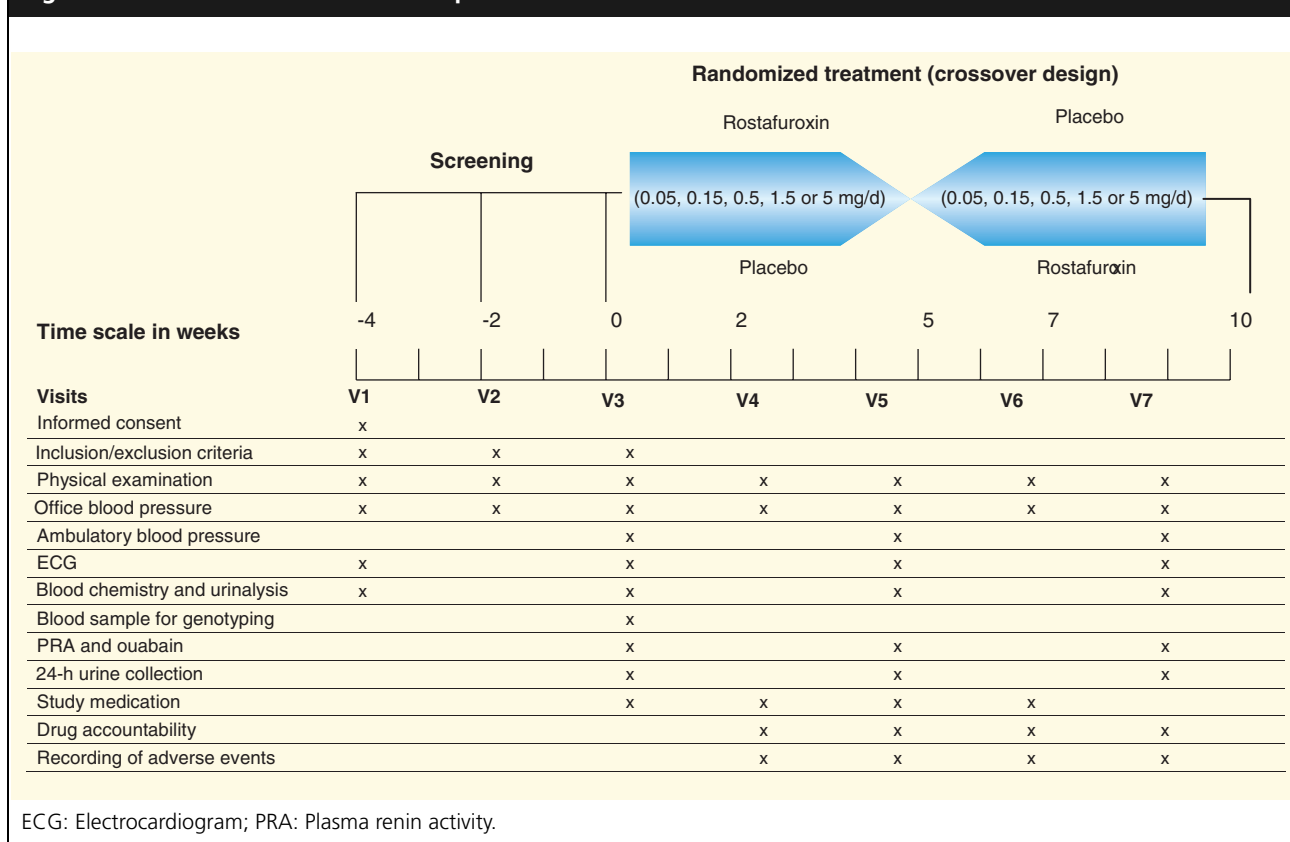
Measurement of plasma renin activity and the serum concentration of endogenous ouabain will be centralized. Local laboratories will perform all other measurements. They should prove compliance with national standards of quality control and provide reference values. The measurements for safety at visit one are not required, if such biochemical analyses were performed within 6 months prior to randomization.

5.5 The 24-h urine collection

At visits three, five and seven, the patients should collect a 24-h urine sample simultaneously with the recording of their 24-h ambulatory blood pressure. They have to use wide-neck containers to separately collect their daytime and night time urine, passing urine directly into the containers. The procedures at visits three, five and seven will be strictly standardized.

- On the day before the clinic visit, patients have to go to the local examination center in the morning after an overnight fast without having taken their study medication. This introductory visit has the following components:
 - First, venous blood will be sampled for the biochemical measurements outlined in section 5.4.
 - Next, the patients have to empty their bladder to provide a fresh urine specimen. This moment marks the start of the urine collection.
 - The investigator initiates the device for ambulatory blood pressure monitoring, as outlined in section 5.3.
 - The investigator witnesses the intake of the study medication by the patient.
 - The investigator notes the starting time of blood pressure monitoring and the urine collection, as well as the time of the intake of the study medication, onto the patient's diary card.
 - The patient then leaves the examination center.
- After the initiation of the urine collection, the patient has to pass all urine into the container for the daytime collection. At night, before going to sleep, the patient has to empty their

Figure 3. Schematic overview of the protocol.



bladder in the daytime container. Thereafter, until rising the next morning, the patient has to pass urine into the night time container. Immediately after rising, the patient has to void a last time in the night time container. All urine produced thereafter has to be collected in the daytime container. Patients log the time of going to bed and getting up onto the diary card.

- On the day of the clinic visit, the patient takes the study and other medication as usual in the morning before breakfast and notes the hour on the diary card.
- On the day of the clinic visit, the patient terminates the urine collection by emptying their bladder into the daytime container. The investigator measures the volume of the daytime and night time urine and sends aliquots of these samples to the local laboratory for the measurement of Na⁺, K⁺ and creatinine, and to the central laboratory for the measurement of aldosterone and the metabolite of rostaduroxin. The investigator terminates the 24-h ambulatory recording and downloads the readings from the device as outlined in section 5.3. They also measure the conventional

blood pressure as described in section 5.1, collect the unused study medication, and perform the other measurements as required for each clinic visit.

5.6 Electrocardiography

At visits one, three, five, and seven, a standard 12-lead electrocardiogram (ECG) should be obtained. ECG recordings in multicenter trials, such as the OASIS-HT, require standardization of the procedures of registration and coding.

5.6.1 Registration of electrocardiograms

All centers will implement similar procedures:

- The ECG room should have an ambient temperature, which is sufficiently high to avoid muscle tremor. The subject, stripped to the waist, is asked to lie on the recording table with the arms relaxed and breathing quietly, and avoiding movements. Subjects whose ECG is taken for the first time should be reassured that there will be no discomfort.
- A calibration mark must be recorded in each channel. If necessary, this calibration mark has to be adjusted to exactly 10 mm with the

ECG filters de-activated. Leads recorded with a reduced calibration (because of high voltage) should be preceded by a calibration mark of exactly 5 mm.

- The tracings are recorded at a paper speed of 25 mm per second. For each lead (I, II, III, aVr, aVI, aVf, and V1–V6) a strip of at least 5 seconds (125 mm) should be recorded.
- If baseline noise or baseline fluctuations occur during the recording, the procedure should be stopped, the patient, the electrodes and the apparatus should be checked, and the ECG recording should be repeated in better conditions.
- Each ECG should contain a complete patient identification (center code, initials, enrolment number, and randomization number). The technician should appropriately label all leads. The physician in charge should review all ECGs. The original ECG has to be clipped onto the patient form. Investigators should keep a duplicate recording in the local patient file.

5.6.2 Phenotypes to be determined from the electrocardiogram

The ECG measurements are relevant to the eligibility of patients and the assessment of drug safety. ECG intervals will be measured in lead II if the T-wave is positive and not deformed by a U-wave. Otherwise, the intervals will be measured in another standard lead or a unipolar lead, in which the QRS interval is widest.

At the screening visit, ECG intervals and QRS voltages should be measured.

- The intervals include atrioventricular conduction time (PR), the depolarization time of the ventricles (QRS), and the electrical systole uncorrected (QT) and corrected (QTc) for heart rate (RR) by Bazett's formula. Patients with a PR-interval of 240 ms or more cannot be randomized.
- The Sokolow-Lyon index is the sum of the S-wave in V1 plus the tallest R-wave in either V5 or V6 [20,22]. Eligible patients should have a Sokolow-Lyon index of less than 38 mm (3.8 mV).
- The Cornell voltage × duration index is computed as the sum of the R-wave in aV1 plus the S-wave in V3 + 8 mm if female, the resultant being multiplied by QRS duration in ms [20,21]. Eligible patients should have an index of less than 2440 mm × ms or 244 mV × ms.

At visits 3, 5 and 7, only the measurements of the ECG intervals should be repeated as part of the evaluation of drug safety.

6. Measurement of genotypes

To our knowledge, OASIS-HT is the first Phase II dose-finding study, which will test a pharmacogenomic hypothesis during the development of a new antihypertensive drug. The DNA will be extracted, stored and genotyped at an academic core laboratory, which is independent of the sponsor (Division of Nephrology and Hypertension, San Raffaele Hospital, Milan, Italy). Genotyping will be carried out on an ABI Prism® 7700 Sequence Detection System (Applied Biosystems, Inc., CA, USA) using a 5' nuclease detection assay.

The statistical analysis of the association between phenotypes and genotypes will be carried out at the Coordinating Office in Leuven, Belgium. The *a priori* genetic hypothesis is that, while accounting for the daily dose of the investigational drug, patient characteristics including sex and age, the 24-h urinary Na⁺ excretion as an index of habitual salt intake, and the plasma concentration of ouabain, the blood pressure lowering activity of rostaduroxin will depend on genetic variation in the adducin subunits and the enzymes involved in the generation of endogenous ouabain.

- Primers and probes for the α -adducin *Gly460Trp* (rs4961 dbSNP), β -adducin *C1797T* (rs4984 dbSNP) and γ -adducin *IVS11+386A>G* (rs3731566 dbSNP) polymorphisms have been described previously [6,28,29]. Screening of the adducin genes is currently in progress and haplotypes will be reconstructed and tested for their functionality before OASIS-HT will report on the genetic results.
- Genetic variation in cholesterol side chain cleavage by cytochrome P450, lanosterol synthase (LSS), 3 β -hydroxy-steroid dehydrogenase (HSD₃B₁) and other enzyme systems implicated in the biosynthesis or metabolism of ouabain will also be measured as potential determinants of the primary and secondary outcomes.

The database generated by the OASIS-HT trial will also create the possibility of running a number of *post hoc* analyses on gene variants not defined at the outset of the trial.

7. Discontinuation of randomized treatment

Patients leaving the randomized part of the trial should be classified as withdrawals or defectors.

The investigators have to document the reasons for stopping randomized treatment in the case report forms.

7.1 Withdrawals

At any time, doctors can withdraw patients from the trial:

- Any condition which, according to the investigator's clinical judgment, puts a patient at risk if the study medication is continued must lead to withdrawal. Reasons for withdrawal are adverse events, unexpected worsening of a patient's clinical condition, the necessity to administer drugs not allowed by the study protocol, uncontrolled hypertension, or orthostatic hypotension.
- Uncontrolled hypertension is a blood pressure of at least 180 mmHg systolic or 110 mmHg diastolic on office measurement, confirmed within one week after the initial measurement. Patients with uncontrolled hypertension must be withdrawn.
- Orthostatic hypotension is a drop in the systolic blood pressure by at least 40 mmHg. The orthostatic change is the difference between the blood pressure measurements in the sitting and standing positions at the clinic visits. Patients experiencing orthostatic hypotension during the first double-blind period should not be withdrawn from the trial, but should be immediately crossed over to the second period. If the event occurs during the second period, the patient will first undergo the end-of-study evaluation and will then be withdrawn.

7.2 Defectors

Patients can also take the initiative to stop randomized treatment.

- The patient can withdraw informed consent or request to stop the study for any reason.
- Patients who discontinue double-blind treatment because of symptoms or adverse events between visits should be invited for a further evaluation at the clinic to fully document the nature of the event.

7.3 Definition & documentation of adverse events

An adverse event is any undesired or unexpected medical event that occurred in a patient during the study, even if there is no apparent relation to the study medication, including any intercurrent illness. Adverse events must be classified as

serious or nonserious. Investigators have to record all adverse events on the case report forms. Patients withdrawn from the trial for an adverse event must be followed up until the final outcome is known. The sponsor and the investigators will comply with international and national regulatory requirements with regard to the reporting of adverse events.

7.3.1 Serious adverse events

Serious adverse events are those that lead to death, are life-threatening, require or prolong hospitalization, cause persistent disability or discomfort, or necessitate an intervention to prevent any of the aforementioned outcomes. Investigators should report serious adverse events immediately to the sponsor.

7.3.2 Nonserious adverse events

All adverse events not meeting the criteria of a serious adverse event should be classified as nonserious. This category also includes elective surgery or hospitalizations planned before the study, but taking place during the trial provided that no condition necessitated advancing the date of the procedure.

8. Statistical methods

Database management will be done with a validated software system that complies with the 21 Code of Federal Regulations [102]. All statistical analyses will be performed with SAS software, version 9.1 (SAS Institute, Inc., NC, USA).

8.1 Sample size

Sample size calculations assumed a standard deviation of systolic blood pressure of 15 mmHg and a drop-out rate of 10%. A total of 440 patients, 88 in each crossover arm, are required to detect, on two-sided tests within each arm of the trial, a mean difference in systolic blood pressure of 8 mmHg between rostauroxin and the placebo with 90% power and 5% significance. The same sample size allows the detection of a mean difference of 5 mmHg in systolic blood pressure between the doses of rostauroxin with 70% power and 5% significance on one-sided tests.

8.2 Patient populations to be analyzed

The statistical analysis will consider three groups of patients:

- Analysis of drug safety will primarily involve all randomized patients who received at least one dose of the double-blind medication.

However, because of the dose-ranging nature of the trial, the safety analysis will be repeated in the group of patients qualifying for the per-protocol analysis.

- The intention-to-treat analysis will include all patients who have at least one evaluation available for each period of double-blind treatment. In the case of missing data, the last observation will be carried forward. Patients dropping out from trial during the first of the two randomized treatment periods will contribute to the analysis with their last measured blood pressure for the first period, which will be compared with the mean blood pressure of the second period of the patients assigned to the same dose of rofuroxin and the same order of rotation of the double-blind medication.
- The per-protocol analysis involves all patients who fulfilled all eligibility criteria, took at least 80% of the prescribed study medication, did not take any drugs prohibited by the protocol, and underwent a complete evaluation at the end of each treatment period. Patients crossed over early during the first double-blind period due to orthostatic hypotension (see section 7.1), or withdrawn during the second phase because of uncontrolled hypertension (see section 7.1), will contribute to the per-protocol analysis with their last blood pressure measurement during each period.

8.3 Analysis of primary and secondary end points

The primary end point consists of the difference in the sitting systolic blood pressure between rofuroxin and placebo, and will be tested by repeated measures analysis of variance within each arm of the trial for each dose of the active drug. If preliminary analyses reveal carry-over effects with a two-sided α -level of 10%, then only the second observation of each crossover period will be used. In a second step of the analysis, the doses of rofuroxin, which compared to the placebo had a significant blood pressure-lowering effect, will be compared with each other for the primary outcome.

Secondary end points measured on a continuous scale, such as the differences in diastolic blood pressure on clinic measurement and those in the 24-hour systolic and diastolic ambulatory blood pressures, will be analyzed in the same manner as the primary end point. The secondary analysis will also include binary blood pressure end points, which will be analyzed by McNemar's test.

- Responders have a decrease in their systolic blood pressure on active treatment by at least 10% of the value on placebo.
- Patients with a normalized blood pressure will have a decrease in their systolic blood pressure on active treatment to a level of less than 135 mmHg.

To determine a responder profile, the continuous and binary blood pressure end points will be subjected to multiple linear and logistic regression. The independent predictors to be considered are sex, age, the 24-h urinary Na⁺ excretion, the plasma concentration of endogenous ouabain, and the variation in the genes encoding the adducin subunits and the enzymes involved in the synthesis of ouabain.

8.4 Analysis of the ambulatory recordings

Daytime and night time are defined on the basis of the short fixed clock-time method [30], with intervals ranging from 10 AM–10 PM and from midnight–6 AM, respectively. Within-subject means of the ambulatory measurements will be computed for 24 h, during daytime and night time, with weighting for the time interval between successive readings [31]. Trough:peak ratios will be computed from diurnal blood pressure profiles, which are synchronized according to the hour of intake of the double-blind medication [32,33]. The trough and peak levels are the blood pressure means over the last time interval of the recording and those within the interval with the maximal reduction in systolic blood pressure, respectively. Initially, the trough:peak ratio will be determined from blood pressure profiles with a 1-h resolution. The effects of smoothing will be investigated by substituting 1-h means by moving (1-h steps) or fixed 2-h intervals [32,33].

8.5 Analysis of safety

While accounting for concurrent medications, the safety and tolerability analysis will involve clinical signs and symptoms, adverse events, laboratory tests on blood and urine, and the ECG measurements. Adverse events will be coded using the *Medical Dictionary for Regulatory Activities* (MedDRA [103]) and medications according to the coding system of the World Health Organization [104]. After conversion to SI units, the biochemical measurements will be normalized to a unique reference range [34]. To facilitate identification of outliers, normalized values at baseline and follow up will be plotted against each other.

For categorical variables, tables will be constructed demonstrating shifts from baseline to follow up.

9. Ethics

OASIS-HT will fully comply with the ethical principles and guidelines for good clinical practice outlined in the Helsinki declaration [35], as well as with all international, European and national regulations. Each participating center received approval for the study from the competent Ethics Committee and National Regulatory Authorities.

10. Discussion

Rostafuroxin selectively displaces ouabain from the Na⁺/K⁺-ATPase receptor and lowers blood pressure in rodent models of hypertension and in hypertensive patients [16,18]. OASIS-HT is an early Phase II dose-finding study of this new compound, in which eligible patients will be randomized to one of five oral doses of rostafuroxin consisting of 0.05, 0.15, 0.5, 1.5, or 5.0 mg/day. Each dose will be compared with a placebo in a double-blind fashion. The decrease in blood pressure is the main outcome variable in terms of efficacy.

To our knowledge, OASIS-HT is the first Phase II dose-finding study in which a genetic hypothesis will be tested. The assumption is that the blood pressure lowering activity of rostafuroxin will depend on the *Gly460Trp* α -adducin polymorphism, which might interact with other genes, such as those encoding the β - and γ -adducin subunits [28,29], the angiotensin-converting enzyme [5,6] or the aldosterone synthase [6]. As extensively reviewed elsewhere [36], rat experiments [1,3], *in vitro* transfection studies [4], interventions in never-treated hypertensive patients [5], and, finally, epidemiologic studies [6,7] revealed a logical sequence of events, leading from a point mutation in the α -subunit [3] to a cellular dysfunction characterized by higher activity of the Na⁺ pump [4]. This in turn led to increased tubular Na⁺ reabsorption in the kidney [5] and, ultimately, hypertension [6]. In Caucasians, the mutated α -adducin allele and the deletion polymorphism of the angiotensin-converting enzyme gene jointly predicted the incidence of hypertension [6]. In cross-sectional analyses of the same population, these two polymorphisms combined were associated with increased femoral intima-media thickness [37], higher serum creatinine concentration [7] and more proteinuria [7].

More recently, the adducin hypothesis was further substantiated by the observation that hypertensive carriers of the mutated α -adducin experienced less cardiovascular complications when they were treated with diuretics instead of drugs that do not antagonize the enhanced tubular Na⁺ reabsorption [38]. Furthermore, in a prospective population study [39], we noticed strong interaction between systolic blood pressure at baseline and the α -adducin polymorphism in relation to the incidence of total and cardiovascular mortality, and all cardiovascular, cardiac and coronary events. For all fatal and nonfatal cardiovascular events combined, the median follow up was 7.2 years [39]. The relative hazard ratio associated with the *460Trp* allele relative to *GlyGly* homozygosity was 2.94 in patients with Grade II systolic hypertension (≥ 160 mmHg) and 0.83 in the other participants. In the former subgroup, the positive predictive value and the attributable risk associated with the *460Trp* allele were 76.9% and 44.3%, respectively [39].

Phase II dose-finding trials commonly implement a parallel-group design, in which patients are randomized to incremental doses of the investigational drug and an untreated control group. The European Project on Genes in Hypertension [28,29,40,41] and several other studies [42–44] demonstrated that phenotype–genotype relations strongly depend on host factors, such as sex and age, as well as on lifestyle and, in particular, salt intake, as reflected by the 24-h urinary excretion of Na⁺. These reports highlighted the concept that phenotype–genotype associations can only be studied within a defined ecogenetic context [28,29,40–44]. Furthermore, previous studies in the general population demonstrated strong interaction between the plasma concentration of ouabain and the 24-h urinary Na⁺ excretion in relation to systolic and diastolic blood pressures [14]. Furthermore, crossover studies compared with parallel-group designs, and ambulatory monitoring compared with the clinic measurement of blood pressure [45], reduce the variability in the estimated effects of any antihypertensive intervention [46,47], and therefore allowed sample size to be economized [46,47]. These considerations explain the design of the OASIS-HT trial, which is a combination of five concurrent crossover studies, one for each dose of rostafuroxin to be studied, and the use of ambulatory monitoring to measure the secondary blood pressure end points.

In 1993, it was observed in 160 volunteers that during the day there was no correlation

between blood pressure and urinary Na⁺ excretion, whereas at night, when the urinary aldosterone:kallikrein ratio fell, the rate of Na⁺ excretion was positively correlated with blood pressure [48]. During sleep, the balance between Na⁺-retention and Na⁺-conservation favors Na⁺ excretion, so that pressure-natriuresis may become more readily evident [48]. In normotensive subjects, Na⁺ excretion is usually maximal in the early afternoon, whereas in hypertensive patients this maximum occurs later during the day [49,50] and plasma volume increases in the afternoon [51]. When the renal capacity to excrete Na⁺ is reduced, such as in carriers of the mutated *460Trp* α -adducin allele [52], Na⁺ excretion might shift to the night time. To detect these possible alterations, the patients enrolled in OASIS-HT will collect separate samples of their daytime and night time urine, while undergoing 24-h ambulatory blood-pressure monitoring.

In a first Phase II study, summarized in the investigators' brochure, mildly hypertensive patients were randomized to losartan (50 mg/day; n = 21) or rosfafuroxin (0.5 mg/day; n = 42). Over 3 months of follow up, both drugs decreased blood pressure, losartan slightly more than rosfafuroxin on a continuous scale, but the numbers of responders or patients with normalized blood pressure were similar in both groups. Among the responders to rosfafuroxin, most had a 24-h urinary Na⁺ excretion of less than 200 mmol/day, whereas patients whose blood pressure did not fall on rosfafuroxin consistently had a Na⁺ output higher than 200 mmol/day (data on file, Sigma-Tau, Pomezia, Italy). In view of these observations, and the interaction between Na⁺ excretion and the plasma concentration of ouabain in a Flemish population [14], patients are only eligible for randomization in OASIS-HT if, in line with current recommendations [19], they observe a moderate reduction in their salt intake, as evidenced by a 24-h urinary Na⁺ excretion of less than 200 mmol at baseline.

From a statistical point of view, OASIS-HT is sufficiently powered to detect, within each arm of the trial, a mean difference in systolic blood pressure of 8 mmHg between rosfafuroxin and placebo on two-sided tests with 90% power and 5% significance. Since the sample size calculations assumed a standard deviation of 15 mmHg for systolic blood pressure, which is more than should be expected in a crossover study, the trial is overpowered for the comparison of the blood pressure changes on rosfafuroxin and placebo. Furthermore, the proposed sample size allows for

the detection of a mean difference of 5 mmHg in systolic blood pressure between the doses of rosfafuroxin with 70% power and 5% significance on one-sided tests. These comparisons involve multiple testing and have a lower statistical power than conventionally used. However, one-sided tests are acceptable for explorative analyses of a secondary end point, although they are usually not recommended.

Rosfafuroxin is the parent compound of a new class of blood pressure-lowering drugs, which undergoes Phase II testing at a time at which most established classes of antihypertensive drugs are going off patent. In view of the multitude of the antihypertensive drugs currently available [19], one might speculate whether there is any need for more compounds. However, hypertension is the age-related disorder that single-handedly is responsible for most drug prescription in the middle-aged and older segments of most developed and developing nations [53]. Any man normotensive at 50 years has a probability of over 90% of becoming hypertensive during the remainder of his lifetime [54]. On the other hand, control rates of hypertension are in general much lower than would be desirable. In the USA, the National Health and Nutrition Examination Survey [55] revealed that the awareness of the hypertensive population improved from 50% in the 1970s to 70% in the 1990s. However, over the same time interval, the proportion of treated hypertensive patients with normalized blood pressure only rose from 10–29% [55]. Recent studies in Europe [56,57] and other parts of the world [58] confirmed that the rule of halves still exists, and that the fractions of hypertensive patients with properly controlled blood pressure range from approximately 5–45%. Even in highly standardized and well-conducted clinical trials, such as the Valsartan Antihypertensive Long-term Use Evaluation (VALUE) study [59], in spite of the systolic blood pressure initiative [60], blood pressure control was not ideal. Indeed, systolic pressure only dropped below 140 mmHg in 64% of the amlodipine-treated patients and in 58% of the group randomized to valsartan [59]. At the end of the trial, only 62 and 56% of the patients reached the predefined blood pressure targets of less than 140 mmHg systolic and 90 mmHg diastolic [59]. Part of these low control rates might be due to the noncompliance of patients and the insufficient motivation of the health professionals to treat hypertension, or to various other factors [61]. Nevertheless, the aforementioned health statistics [54–58] illustrate that new and efficacious antihypertensive drugs with a

Highlights

- Recent experimental, clinical and epidemiological studies strongly support an interactive role of mutated α -adducin, sodium (Na⁺)/potassium(K⁺)-adenosine triphosphatase (ATPase) activity, and endogenous ouabain in the regulation of Na⁺ homeostasis and the pathogenesis of hypertension.
- Rostafuroxin is a new antihypertensive compound that antagonizes the pressor effect of ouabain and normalizes ouabain-dependent upregulation of the renal Na⁺/K⁺ pump.
- The Ouabain and Adducin for Specific Intervention on Sodium in HyperTension (OASIS-HT) trial is an ongoing Phase II dosage study of rostafuroxin.
- This trial will also test the pharmacogenomic hypothesis that the blood pressure lowering activity of rostafuroxin will depend on genetic variation in the adducin cytoskeleton proteins and in the enzymes involved in the biosynthesis of ouabain.
- High-fidelity phenotyping is a cornerstone to the research goals of OASIS-HT.
- A pharmacogenomic approach will be a critical step in moving the adducin hypothesis from experimental studies to clinical application.
- New and efficacious antihypertensive drugs with a mechanism of action different from the existing classes, and characterized by a low incidence of side-effects, would be a welcome addition to the therapeutic armamentarium.

mechanism of action different from the existing classes, and characterized by a low incidence of side effects, would be a welcome addition to the therapeutic armamentarium, especially if hypertensive patients likely to respond can be readily identified by genetic stratification.

11. Conclusion

OASIS-HT is a Phase II dose-finding trial of rostafuroxin, an ouabain analog, which is the parent compound of a new class of antihypertensive

drugs. In a double-blind fashion, this study will compare the blood pressure-lowering efficacy of the active compound administered in a 100-fold dosing range and that of a placebo. The wide dosing range accounts for the preclinical studies, which demonstrated that the effects of rostafuroxin mediated via ouabain occur at a dose or concentration approximately ten-times lower than those interfering with mutated α -adducin [17,18].

The enrolment of centers started on January 1, 2005. The first patient was randomized on April 6, 2005. On July 31, 2005, 17 of the 39 centers had obtained ethical and regulatory approval. Of 127 patients screened, 94 were still going through the run-in period, 33 had failed to meet the entry criteria, 82 had been randomized, 53 were continuing double-blind follow-up, and 29 had completed the trial. If these rates of screening, randomization and follow-up are sustained, OASIS-HT should achieve full recruitment and follow up by the end of 2005, and report within 6 months of this milestone.

Acknowledgments

Sigma-Tau (Pomezia, Italy) sponsors the OASIS-HT trial. The authors acknowledge the expert assistance of Sandra Covens, Sylvia Van Hulle, and Renilde Wolfs (Leuven, Belgium). The Study Coordinating Centre (Leuven, Belgium) receives financial support from Sigma-Tau within the legal framework of Leuven Research and Development [105] for the scientific management of the OASIS-HT trial. Giuseppe Bianchi is an adviser to the Prassis Research Institute (Settimo Milanese, Milan, Italy), which is affiliated with Sigma-Tau.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Ferrandi M, Bianchi G: Genetic mechanisms underlying the regulation of urinary sodium excretion and arterial blood pressure: the role of adducin. *Acta Physiol Scand.* 168, 187–193 (2000).
2. Matsuoka Y, Li X, Bennett V: Adducin: structure, function and regulation. *Cell Mol Life Sci.* 57, 884–895 (2000).
- This review paper comprehensively describes current knowledge on the structure, function and regulation of adducin.
3. Bianchi G, Tripodi G, Casari G *et al.*: Two point mutations within the adducin genes are involved in blood pressure variation: *Proc Natl Acad Sci USA* 91, 3999–4003 (1994).
- This original report shows that adducin polymorphism is associated with blood pressure variation in Milan rats.
4. Tripodi G, Valtorta F, Torielli L *et al.*: Hypertension-associated point mutations in the adducin α and β subunits affect actin cytoskeleton and ion transport. *J Clin Invest.* 97, 2815–2822 (1996).
5. Barlassina C, Schork NJ, Manunta P *et al.*: Synergistic effect of α -adducin and ACE genes causes blood pressure changes with body sodium and volume expansion. *Kidney Int.* 57, 1083–1090 (2000).
6. Staessen JA, Wang JG, Brand E *et al.*: Effects of three candidate genes on prevalence and incidence of hypertension in a Caucasian population. *J Hypertens.* 19, 1349–1358 (2001).
7. Wang JG, Staessen JA, Tizzoni L *et al.*: Renal function in relation to three candidate genes. *Am J Kidney Dis.* 38, 1158–1168 (2001).
8. De Angelis C, Hauptert GT Jr: Hypoxia triggers release of an endogenous inhibitor of Na⁺-K⁺-ATPase from midbrain and adrenal. *Am J Physiol.* 274, F182–F188 (1998).
9. Rossi G, Manunta P, Hamlyn JM *et al.*: Immunoreactive endogenous ouabain in primary aldosteronism and essential hypertension: relationship with plasma renin, aldosterone and blood pressure levels. *J Hypertens.* 13, 1181–1191 (1995).
10. Manunta P, Messaggio E, Ballabeni C *et al.*: Plasma ouabain-like factor during acute and chronic changes in sodium balance in essential hypertension. *Hypertension* 38, 198–203 (2001).
11. Orlov SN, Adragna NC, Adarichev VA, Hamet P: Genetic and biochemical determinants of abnormal monovalent ion transport in primary hypertension. *Am J Physiol.* 276, C511–C536 (1999).

12. Schoner W: Endogenous cardiac glycosides, a new class of steroid hormones. *Eur. J. Biochem.* 269, 2440–2448 (2002).
13. Anderson RGW, Jacobson K: A role for lipid shells in targeting proteins to caveolae, raft, and other lipid domains. *Science* 296, 1821–1825 (2002).
14. Wang JG, Staessen JA, Messaggio E *et al.*: Salt, endogenous ouabain and blood pressure in the general population. *J. Hypertens.* 21, 1475–1481 (2003).
15. Quadri L, Bianchi G, Cerri A *et al.*: 17 β -(3-furyl)-5 β -androstane-3 β ,14 β ,17 α -triol (PST 2238). A very potent antihypertensive with a novel mechanism of action. *J. Med. Chem.* 40, 1561–1564 (1997).
16. Ferrari P, Ferrandi M, Tripodi G *et al.*: PST 2238: a new antihypertensive compound that modulates Na,K-ATPase in genetic hypertension. *J. Pharmacol. Exp. Ther.* 288, 1074–1083 (1999).
- **This original report describes the *in vitro* and *in vivo* pharmacologic characteristics of a new antihypertensive molecule that selectively interacts with the Na⁺/K⁺-ATPase and antagonizes the pressor effect of ouabain.**
17. Ferrandi M, Molinari I, Barassi P, Minotti E, Bianchi G, Ferrari P: Organ hypertrophic signaling within caveolae membrane subdomains triggered by ouabain and antagonized by PST2238. *J. Biol. Chem.* 279, 33306–33314 (2004).
18. Ferrari P, Ferrandi M, Torielli L, Tripodi G, Melloni P, Bianchi G: PST2238, a new antihypertensive compound that modulates Na⁺,K⁺-ATPase and antagonizes the pressor effect of OLF. *Cardiovasc. Drug Rev.* 17, 39–57 (1999).
19. European Society of Hypertension/European Society of Cardiology Guidelines Committee: 2003 European Society of Hypertension/European Society of Cardiology Guidelines for the Management of Arterial Hypertension. *J. Hypertens.* 21, 1011–1053 (2003).
- **These guidelines have been prepared on the basis of the best available evidence for all key recommendations for the management of arterial hypertension, and with the principle that guidelines should be educational rather than merely prescriptive.**
20. Casale PN, Devereux RB, Alonso DR, Campo E, Kligfield P: Improved sex-specific criteria of left ventricular hypertrophy for clinical and computer interpretation of electrocardiograms: validation with autopsy findings. *Circulation* 75, 565–572 (1987).
21. Okin PM, Roman MJ, Devereux RB, Kligfield P: Electrocardiographic identification of increased left ventricular mass by simple voltage-duration products. *J. Am. Coll. Cardiol.* 25, 417–423 (1995).
22. Sokolow M, Lyon TP: The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. *Am. Heart. J.* 37, 161–186 (1949).
23. O'Brien E, Asmar R, Beilin L *et al.*: European Society of Hypertension recommendations for conventional, ambulatory and home blood pressure measurement. *J. Hypertens.* 21, 821–848 (2003).
24. O'Brien E, Pickering T, Asmar R *et al.*: Working group on blood pressure monitoring of the European Society of Hypertension International Protocol for validation of blood pressure measuring devices in adults. *Blood Press. Monit.* 7, 3–18 (2002).
25. O'Brien E, Mee F, Atkins N, O'Malley K: Accuracy of the SpaceLabs 90207 determined by the British Hypertension Society Protocol. *J. Hypertens.* 9(Suppl. 5), S25–S31 (1991).
26. White WB, Berson AS, Robbins C *et al.*: National standard for measurement of resting and ambulatory blood pressures with automated sphygmomanometers. *Hypertension* 21, 504–509 (1993).
27. O'Brien E, Petrie J, Littler WA *et al.*: The British Hypertension Society protocol for the evaluation of blood pressure measuring devices. *J. Hypertens.* 11(Suppl. 2), S43–S63 (1993).
28. Tikhonoff V, Kuznetsova T, Stolarz K *et al.*: β -Adducin polymorphisms, blood pressure and sodium excretion in three European populations. *Am. J. Hypertens.* 16, 840–846 (2003).
29. Cwynar M, Staessen JA, Tichá M *et al.*: Epistatic interaction between α - and γ -adducin influences peripheral and central pulse pressure in White Europeans. *J. Hypertens.* 23, 961–969 (2005).
30. Fagard RH, Staessen JA, Thijs L: Optimal definition of daytime and night-time blood pressure. *Blood Press. Monit.* 2, 315–321 (1997).
31. Thijs L, Staessen J, Fagard R: Analysis of the diurnal blood pressure curve. *High Blood Press.* 1, 17–28 (1992).
32. Staessen JA, Thijs L, Byttebier G *et al.*: Determining the trough-to-peak ratio in parallel-group trials. *Hypertension* 29, 659–667 (1997).
33. Staessen JA, Bieniaszewski L, Buntinx F *et al.*: The trough-to-peak ratio as an instrument to evaluate antihypertensive drugs. *Hypertension* 26, 942–949 (1995).
34. Chuang-Stein C: Some issues concerning the normalization of laboratory data based on reference ranges. *Drug Inform. J.* 35, 153–156 (2000).
35. 41st World Medical Assembly: Declaration of Helsinki: recommendations guiding physicians in biomedical research involving human subjects. *Bull. Pan. Am. Health Organ.* 24, 606–609 (1990).
36. Bianchi G, Ferrari P, Staessen JA: Adducin polymorphism. Detection and impact on hypertension and related disorders. *Hypertension* 45, 331–340 (2005).
- **This brief review provides detailed information on genetic variation in the adducin genes and how it affects the pathogenesis of cardiovascular disease.**
37. Balkestein EJ, Wang JG, Struijker Boudier HAJ *et al.*: Carotid and femoral intima-media thickness in relation to three candidate genes in a Caucasian population. *J. Hypertens.* 20, 1551–1561 (2002).
38. Psaty BM, Smith NL, Heckbert SR *et al.*: Diuretic therapy, the α -adducin gene variant, and the risk of myocardial infarction or stroke in persons with treated hypertension. *JAMA* 287, 1680–1690 (2002).
39. Li Y, Thijs L, Kuznetsova T *et al.*: Cardiovascular risk in relation to α -adducin Gly460Trp polymorphism and systolic pressure. A prospective population study. *Hypertension* 46(3), 527–532 (2005).
- **This original report shows that mutation of the α -adducin gene in association with systolic blood pressure leads to cardiovascular complications in the general population.**
40. Stolarz K, Staessen JA, Kuznetsova T *et al.*: Host and environmental determinants of heart rate and heart rate variability in four European populations. *J. Hypertens.* 21, 525–535 (2003).
41. Kuznetsova T, Staessen JA, Brand E *et al.*: Sodium excretion as modulator of genetic associations with cardiovascular phenotypes in the European Project on Genes in Hypertension. *J. Hypertens.* (2005) (In press).
42. Dumas P, Kren V, Krenová D, Pravenec M, Hamet P, Tremblay J: Identification and chromosomal localization of exogenous components of electrolyte excretion. *J. Hypertens.* 20, 209–217 (2002).
43. Cooper RS: Gene–environment interactions and the etiology of complex disease. *Ann. Intern. Med.* 139, 437–440 (2003).

44. Schut AFC, Bleumink GS, Stricker BHC *et al.*: Angiotensin converting enzyme insertion/deletion polymorphism and the risk of heart failure in hypertensive subjects. *Eur. Heart J.* 25, 2143–2148 (2004).
45. Staessen JA, O'Brien ET, Thijs L, Fagard RH: Modern approaches to blood pressure measurement. *Occup. Environ. Med.* 57, 510–520 (2000).
46. Staessen JA, Thijs L, Parati G, Mancia G, O'Brien ET, on behalf of the Syst-Eur Investigators: Clinical trials with ambulatory blood pressure monitoring: fewer patients needed? *Lancet* 344, 1552–1556 (1994).
47. Friedman JM, Furberg CD, DeMets DL: Basic study design. In: *Fundamentals of Clinical Trials*, 2nd ed. Fundamentals of Clinical Trials, PSG Publishing Company Inc., Littleton, Massachusetts, USA, 33–50 (1985).
48. Staessen JA, Birkenhäger W, Bulpitt CJ *et al.*: The relationship between blood pressure and sodium and potassium excretion during the day and at night. *J. Hypertens.* 11, 443–447 (1993).
49. Kawasaki T, Ueno M, Uezono K *et al.*: The renin–angiotensin–aldosterone system and circadian rhythm of urine variables in normotensive and hypertensive subjects. *Jap. Circ. J.* 48, 168–172 (1984).
50. Bultasova H, Veselkova A, Brodan V, Pinsker P: Circadian rhythms of urinary sodium, potassium and some agents influencing their excretion in young borderline hypertensives. *Endocrinol. Exp.* 20, 359–369 (1986).
51. Parry EHO, Dollery CT: Diurnal changes in plasma volume in hypertensive patients: effect of posture and sodium depletion. *Clin. Sci.* 35, 373–380 (1968).
52. Manunta P, Cusi D, Barlassina C *et al.*: α -Adducin polymorphisms and renal sodium handling in essential hypertensive patients. *Kidney Int.* 53, 1471–1478 (1998).
53. Staessen JA, Wang J, Bianchi G, Birkenhäger WH: Essential hypertension. *Lancet* 361, 1629–1641 (2003).
- **This review paper comprehensively describes current knowledge on the epidemiology, pathophysiology, genetics, diagnosis, and treatment of essential hypertension.**
54. Vasan RS, Beiser A, Larson MG, Kannel WB, D'Agostino RB, Levy D: Residual lifetime risk of developing hypertension in middle-aged women and men. The Framingham Heart Study. *JAMA* 287, 1003–1010 (2002).
55. Burt VL, Cutler JA, Higgins M *et al.*: Trends in the prevalence, awareness, treatment, and control of hypertension in the adult US population. Data from the Health Examination Surveys, 1960 to 1991. *Hypertension* 26, 60–69 (1995).
56. Staessen JA, Kuznetsova T, Stolarz K: Hypertension prevalence and stroke mortality across populations. *JAMA* 289, 2420–2422 (2003).
57. Weinehall L, Öhgren B, Persson M *et al.*: High remaining risk in poorly treated hypertension: the 'rule of halves' still exist. *J. Hypertens.* 20, 2081–2088 (2002).
58. Cruickshank JK, Mbanya JC, Wilks R *et al.*: Hypertension in four African-origin populations: current 'Rule of Halves', quality of blood pressure control and attributable risk of cardiovascular disease. *J. Hypertens.* 19, 41–46 (2001).
59. Julius S, Kjeldsen SE, Weber M *et al.*: Outcomes in hypertensive patients at high cardiovascular risk treated with valsartan- or amlodipine-based regimens: VALUE, a randomised trial. *Lancet* 363, 2022–2031 (2004).
60. Julius S, Kjeldsen SE, Brunner H *et al.*: VALUE trial: long-term blood pressure trends in 13,449 patients with hypertension and high cardiovascular risk. *Am. J. Hypertens.* 16, 544–548 (2003).
61. Staessen JA, Thijs L, Birkenhäger WH: Noncardiovascular illness as barrier to antihypertensive treatment. *Hypertension* 46, 255–256 (2005).

Websites

101. www.dableducational.org dabl® Educational Trust website.
102. www.fda.gov/cder/offices/ods/regs.htm Center for Drug Evaluation and Research website.
103. www.meddransso.com Medical Dictionary for Regulatory Activities website.
104. www.umc-products.com The UMC website.
105. www.kuleuven.be/lrd/about/mission.html K.U.Leuven R&D website.

Appendix

Participating centers and coordination

Participating centers**Belgium**

Celis H (Leuven)

The Czech Republic

Cifková R (Prague)

Filipovský J (Pilsen)

Peleška J (Prague)

Widimský J Jr (Prague)

Souček M (Brno)

Vítovec J (Brno)

Špinar J (Brno)

Stanek V (Prague)

Widimský J Sr (Prague)

France

Fournier A (Amiens)

Germany

Brand E (Münster)

Brand-Herrmann SM (Münster)

Ireland

O'Brien ET (Dublin)

Stanton A (Dublin)

Italy

Basso G (Schio)

Casiglia E (Padua)

Tikhonoff V (Padua)

Colangeli G (Castelfranco)

Degli Esposti E (Ravenna)

Glorioso N (Sassari)

Manunta P (Milan)

Timio M (Foligno)

The Netherlands

de Leeuw PW (Maastricht)

Krekels M (Sittard)

Poland

Dluzniewski M (Warsaw)

Grodzicki T (Krakow)

Januszewicz A (Warsaw)

Kawecka- Jaszcz K (Krakow)

Olszanecka A (Krakow)

Stolarz K (Krakow)

Klocek M (Krakow)

Narkiewicz K (Gdansk)

Wyrzykowski B (Gdansk)

Piwowarska W (Krakow)

Tykarski A (Poznan)

The Russian Federation

Nikitin Y (Novosibirsk)

Slovenia

Brugljan J (Ljubljana)

Acceto R (Ljubljana)

Spain

Armario P (Barcelona)

Calvo Gomez C (Santiago de Compostela)

Division Garrote JA (Salud)

Coca Payeras A (Barcelona)

Hernández Menárguez F (Murcia)

Luque Otero M (Madrid)

Pascual Izuel M (Sagunto)

Pose Reino A (Santiago de Compostela)

Redón J (Valencia)

Switzerland

Burnier M (Lausanne)

United Kingdom

McInnes G (Glasgow)

Coordination**Project Coordinator**

Staessen JA

Scientific Coordinator

Bianchi G

National Coordinators

Acceto R (Slovenia)

Bianchi G (Italy)

Brand E (Germany)

Burnier M (Switzerland)

Celis H (Belgium)

Filipovský J (Czech Republic)

Fournier A (France)

Kawecka Jaszcz K (Poland)

McInnes G (United Kingdom)

Nikitin Y (Russian Federation)

O'Brien ET (Ireland)

Redón J (Spain)

Study Coordinating Office (Leuven, Belgium)

Kuznetsova T

Plusquin M

Staessen JA

Thijs L

Logistic Coordination (Pomezia, Italy)

Bacchieri A