Trial Title	Renin Genotype and Response to Renin Angiotensin System Blockade
EudraCT Number	2008-003568-20
Study Ref. No.	RGR 001
Sponsor	Prof. Alice Stanton
Date of Report	30th November 2021

CONFIDENTIAL

Signature pages for clinical trial report

I have read this report and confirm that to the best of my knowledge it accurately describes the conduct and results of the study.

Signed: 

Date: 30/11/2021

Print name: Prof. Alice Stanton
Position: Principal Investigator and Sponsor
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1 TITLE PAGE

Study title:	Renin Genotype and Response to Renin Angiotensin System Blockade
Name of test drugs:	Aliskiren 300 mg daily, Candesartan 32 mg daily, Perindopril 10 mg daily. Amlodipine 10 mg (concomitant medication)
Indication studied:	Hypertension
Study description:	A phase IV single centre prospective randomized open cross-over pharmacogenetic clinical trial comparing BP lowering responses to a renin inhibitor (aliskiren), an angiotensin receptor blocker (candesartan), and an angiotensin converting enzyme inhibitor (perindopril), amongst REN-5312 T allele carriers and amongst REN-5312 CC homozygotes.
Sponsor:	Prof. Alice Stanton
Protocol:	Version 3 dated July 29th 2008
Clinical Phase:	Phase IV
Study dates:	First patient first visit: August 8th 2008 Last patient last visit: June 30th 2011

Investigators:	Principle Investigator Address:	Prof. Alice Stanton RCSI Clinical Research Centre, Royal College of Surgeons in Ireland, RCSI Education & Research Centre, Beaumont Hospital, Beaumont, Dublin 9, Ireland.
	Co-Investigators	Prof. Brendan McAdam Department of Cardiology, Beaumont Hospital, Dublin 9, Ireland. Dr. Ursula Quinn, Department of Cardiology, Beaumont Hospital, Dublin 9, Ireland.
Medical Monitor:	Ms. Deirdre Hyland	

GCP Statement:	This study was performed in compliance with ICH Good Clinical Practice (GCP) including the archiving of essential documents
Date of report:	30th November 2021

2 SYNOPSIS

Title of Study	Renin Genotype and Response to Renin Angiotensin System Blockade
Investigational Medicinal Product(s)	Aliskiren Candesartan Perindopril Amlodipine
Phase:	Phase IV
Investigator(s)	Prof. Alice Stanton Prof. Brendan McAdam Dr. Ursula Quinn,
Study centre(s)	Department of Cardiology, Blood Pressure Unit, and RCSI Clinical Research Centre, Beaumont Hospital, Dublin 9, Ireland.
Study period	First patient first visit: August 8th 2008 Last patient last visit: June 30th 2011
Date of final analysis	27th September 2016
Objectives	To confirm that genotyping of the REN-5312C/T polymorphism identifies individuals who have different BP lowering responses to particular blockers of the renin angiotensin system, and therefore validate REN-5312C/T genotyping as a useful pharmacogenetic assay. Specific primary objectives were: 1. To determine the relationship between REN-5312C/T genotype and plasma renin activity (PRA); 2. To confirm (or refute) that genotyping of the REN-5312C/T polymorphism, alone or in combination with plasma renin activity (PRA), predicts BP lowering responses to renin-angiotensin system (RAS) blockade.
Methodology	A phase IV single centre prospective randomized open cross-over pharmacogenetic clinical trial comparing BP lowering responses to a renin inhibitor (aliskiren), an angiotensin receptor blocker (candesartan), and an angiotensin converting enzyme inhibitor (perindopril), amongst REN-5312 T allele carriers and amongst REN-5312 CC homozygotes.
Number of patients	Planned: 100 Analysed: 98
Main criteria for inclusion	• Male or female outpatients • Age greater than 18 years. • Written informed consent provided. • Patients with essential hypertension who are either antihypertensive treatment naïve, or taking a maximum of two antihypertensive agents. • Baseline mean 24-hour systolic pressure > 130 mm Hg, and < 160 mm Hg, either on no treatment or on amlodipine 10 mg daily.

Test product, dose and mode of administration	Aliskiren 300 mg daily, Candesartan 32 mg daily, Perindopril 10 mg daily. Amlodipine 10 mg (concomitant medication) All oral administration

Duration of treatment	4 weeks on each of 3 treatments
Criteria for evaluation	The primary efficacy end-point was the change from baseline in 24-hour systolic BP with each drug treatment. Secondary efficacy end-points were; • change from baseline in 24-hour diastolic BP • change from baseline in daytime systolic BP • change from baseline in daytime diastolic BP • change from baseline in night-time systolic BP • change from baseline in night-time diastolic BP Safety endpoints; • Adverse events • Adverse drug reactions • Worsening of hypertension • Symptomatic hypotension • Electrolyte abnormalities • Deterioration in renal function (serum creatinine and creatinine clearance)
Statistical methods	The principal hypothesis tested within this clinical trial was whether REN-5312C/T genotype, alone or in combination with PRA, provides improved prediction of BP lowering responses to RAS blockade, additional to that provided by drug type, age, and baseline BP. Generalised linear modelling (stepwise regression models with backward elimination) was used to test for independent predictors of BP lowering responses. The stepwise regression was performed by iteratively removing non-significant ($p<0.05$) variables from the model until only variables significantly associated with BP lowering responses remained.

SUMMARY CONCLUSIONS	
Efficacy Results	Blood pressure lowering responses were observed to be greater: • With candesartan treatment compared with aliskiren or perindopril treatments; • In females compared to males; • In participants with higher blood pressures; • In participants with higher PRA levels (supine PRA of ≥ 1ng/mL/hour); • In REN-5312T allele carriers who also had higher BP levels and/or lower PRA levels (supine PRA of ≤ 1ng/mL/hour). Additional findings included: • An association between REN-5312C/T genotype and PRA; • An association between REN-5312C/T genotype and serum potassium levels
Safety Results:	There were no safety concerns identified within this trial. There were eight study withdrawals during the study period. The 8 study withdrawals that occurred during the study period were in large part associated with candesartan induced hypotension. There were two reported Serious Adverse Events during the study period, both of which were deemed unrelated to study participation.

Conclusion	REN-5312C/T may become a genotype used in practice to identify patients who will gain maximal benefit from RAS blockade both in terms of blood pressure lowering and protection from end-organ damage. However it is doubtful that the REN-5312C/T genotype alone will act as a useful predictor of antihypertensive response. It is much more likely that REN-5312C/T will direct treatment as one of a panel of genes identified as having an impact on prediction of response to antihypertensive therapy.
Date of the Report	30th November 2021

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4 LIST OF ABBREVIATIONS & DEFINITION OF TERMS

ABPM	Ambulatory Blood Pressure Monitor
ACE	Angiotensin Converting Enzyme
ACEi	Angiotensin Converting Enzyme Inhibitor
AGT	Angiotensinogen
Ang I	Angiotensin I
Ang II	Angiotensin II
ANOVA	Analysis of Variance
AT1R	Angiotensin Type-1 Receptor
ARB	Angiotensin Receptor Blocker
BMI	Body Mass Index
BP	Blood Pressure
CAD	Coronary Artery Disease
CCB	Calcium Channel Blocker
cDNA	Complementary Deoxyribonucleic Acid
CHD	Coronary Heart Disease
DBP	Diastolic Blood Pressure
DNA	Deoxyribonucleic Acid
DRI	Direct Renin Inhibitor
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
ESC	European Society of Cardiology
ESH	European Society of Hypertension
GWAS	Genome Wide Association Studies
HR	Heart Rate
Hr	Hour
ICH GCP	International Conference on Harmonisation, Good Clinical Practice
IMB	Irish Medicines Board
I/D	Insertion/Deletion Polymorphism
kDa	Kilodalton
Kg	Kilograms
mg	Milligrams
mL	Millilitres
mm Hg	Millimetres of Mercury (Mercury, Hg)
mmol/L	Millimoles per Litre
mRNA	Messenger Ribonucleic Acid
MR	Mineralocorticoid Receptor

ng	Nanograms
NICE	National Institute for Clinical Excellence
PBS	Phosphate-buffered Saline
PCR	Polymerase Chain Reaction
PRA	Plasma Renin Activity
RAS	Renin Angiotensin System
RAAS	Renin Angiotensin Angiotensinogen System
RIA	Radioimmunoassay
rRNA	Ribosomal Ribonucleic Acid
rpm	Revolutions Per Minute
RNA	Ribonucleic Acid
SAEs	Serious Adverse Events
SBP	Systolic Blood Pressure
SEM	Standard Error of Mean
SLR	Specified Laboratory Reagent
SNS	Sympathetic Nervous System
SNP	Single Nucleotide Polymorphism
STEMI	ST Segment Elevation Myocardial Infarction
tSNP	Tagging Single Nucleotide Polymorphism
UK	United Kingdom
US	United States
WHO	World Health Organisation
µL	Microlitre
µmol	Micromolar

5 ETHICS AND REGULATORY APPROVAL

Independent Ethics Committee Approval

Approval was obtained from Beaumont Hospital Medical Research Ethics Committee (REC reference 08/60, date of approval 7th August 2008, Protocol Version 3 RGR001) prior to study recruitment.

Regulatory Approval

The study gained full regulatory approval from the Irish Medicines Board (Case number 2051960, date of approval 2nd June 2008).

Ethical Conduct of the Study

The study was performed in accordance with the current version of the declaration of Helsinki (2013), and in compliance with the International Conference on Harmonization (ICH) guidelines on Good Clinical Practice (GCP)

Patient Information and Consent

Written informed consent was obtained from all participants prior to entry into the study. In obtaining informed consent the study investigator provided the potential participant with information about the purposes, methods, possible risks and benefits of participating in the study. All potential participants had an opportunity to discuss the study with study staff. Participation was voluntary, and all participants were free to withdraw at any time.

Copies of the;

- Study Protocol
- Participant Information Leaflet
- Participant Consent Forms
- Ethics Committee Approval
- Regulatory Approval

are to be found at appendices 14.1 -14.5 respectively.

6 INTRODUCTION

The renin-angiotensin system (RAS) has a central role in the regulation of blood pressure level. International guidelines (US, European and UK) all advocate the use of agents which block varying steps of the RAS as first-line or second-line therapy for hypertension. The most studied candidate genes in terms of contribution to hypertension are those coding for the renin-angiotensin aldosterone system (Redon et al. 2004). Polymorphisms within the Renin-Angiotensin Aldosterone System (RAAS) such as the ACE I/D, angiotensinogen (AGT) M235T polymorphisms and angiotensin II type-1 receptor (AT1R) polymorphism A1166C have all been well characterized.

Renin is an aspartic protease and is the rate-limiting step in the process that ultimately results in angiotensin II production. The renin gene, REN, spans 12kB of DNA and contains 8 introns (Hobart et al. 1984). It is located at 1q32 (Cohen-Haguenauer et al. 1989). A common single nucleotide polymorphism (SNP) in a renin distal enhancer element (REN-5312C/T, rs12750834) has been reported to influence in vitro gene transcription in transfected human choriodecidual cells (Germain et al. 1998). In 2002, Fuchs and colleagues noted 45% greater rates of renin gene transcription in the presence of a -5312T allele rather than a -5312C allele (Fuchs et al. 2002). A further study in diseased human kidney observed greater renin gene expression in T-allele carriers (Makino et al. 2015). We recently replicated the finding of increased renin gene expression associated with carriage of the -5312T allele (unpublished data).

The SNP has been shown to have in vivo functional activity in humans, with carriage of the -5312 T-allele associated with elevated ambulatory and clinic BP levels in a cohort of healthy bank employees (Moore et al. 2007). This was replicated in a second population (Vangjeli et al. 2010).

It also appeared that presence of the polymorphism predicted BP lowering responses to RAS blockade in hypertensive patients, independent of plasma renin activity, (PRA) (Moore et al. 2007). Aliskiren, a direct renin inhibitor, when compared with losartan, an angiotensin receptor blocker, was found to have a significantly greater nocturnal BP lowering amongst -5312 CC homozygotes than T-allele carriers. The reverse was true for losartan, with T-allele carriers achieving BP lowering twice that of CC homozygotes. Thus REN-5312 C/T carriership could have potential use as a pharmacogenetic assay (Moore et al. 2007).

7 STUDY OBJECTIVES

To confirm that genotyping of the REN-5312C/T polymorphism identifies individuals who have different BP lowering responses to particular blockers of the renin angiotensin system, and therefore validate REN-5312C/T genotyping as a useful pharmacogenetic assay.

Specific primary objectives were:

1. To determine the relationship between REN-5312C/T genotype and plasma renin activity (PRA);
2. To confirm (or refute) that genotyping of the REN-5312C/T polymorphism, alone or in combination with plasma renin activity (PRA), predicts BP lowering responses to renin-angiotensin system (RAS) blockade.

Drug class and potency, age, ethnicity and baseline BP are currently regarded as clinically useful predictors of BP lowering responses to antihypertensive therapy (Chapman et al. 2002). Hence the principal hypothesis to be tested in this trial was whether REN-5312C/T genotype would predict blood pressure lowering, and whether addition of PRA would yield additive

8 STUDY METHODS

Study Design

This was a phase IV single centre prospective randomized open cross-over pharmacogenetic clinical trial comparing BP lowering responses in hypertensive individuals to a renin inhibitor (Aliskiren), an angiotensin receptor blocker (Candesartan), and an angiotensin converting enzyme inhibitor (Perindopril) according to age, baseline BP, PRA level, and REN -5312 C/T genotype.

The primary endpoint was the change from baseline in mean 24-hour systolic blood pressure with each drug therapy. Secondary endpoints included the changes from baseline in daytime systolic, night-time systolic, 24-hour diastolic, daytime diastolic and night-time diastolic blood pressures with the three drug therapies.

Participants were reviewed at the Blood Pressure Unit, Beaumont Hospital and at the Clinical Research Centre, Beaumont Hospital. At the screening visit, a comprehensive medical history and physical examination was performed. Individuals not taking any antihypertensive medications were booked for their randomisation visit. Those individuals on antihypertensive medications were counselled regarding discontinuing their treatment for a one-week period. After one week free of all vasoactive medications clinic BP and 24-hour ambulatory blood pressures were measured and phlebotomy performed.

Participants whose baseline mean 24-hour systolic pressure was greater than 130 mm Hg, and less than 160 mm Hg off all antihypertensive treatment were eligible for inclusion. Those, whose baseline mean 24-hour systolic pressure was greater than 160 mm Hg and less than 175 mm Hg, were prescribed amlodipine 10 mg daily, and 24-hour ambulatory BP monitoring was repeated 2 weeks later. Once their mean 24-hour systolic pressure was then greater than 130 mm Hg, and less than 160 mm Hg on amlodipine 10mg, these participants were eligible for inclusion. For these patients, treatment with amlodipine continued throughout the clinical trial alongside each of the renin-angiotensin system blockers (“dual-therapy”).

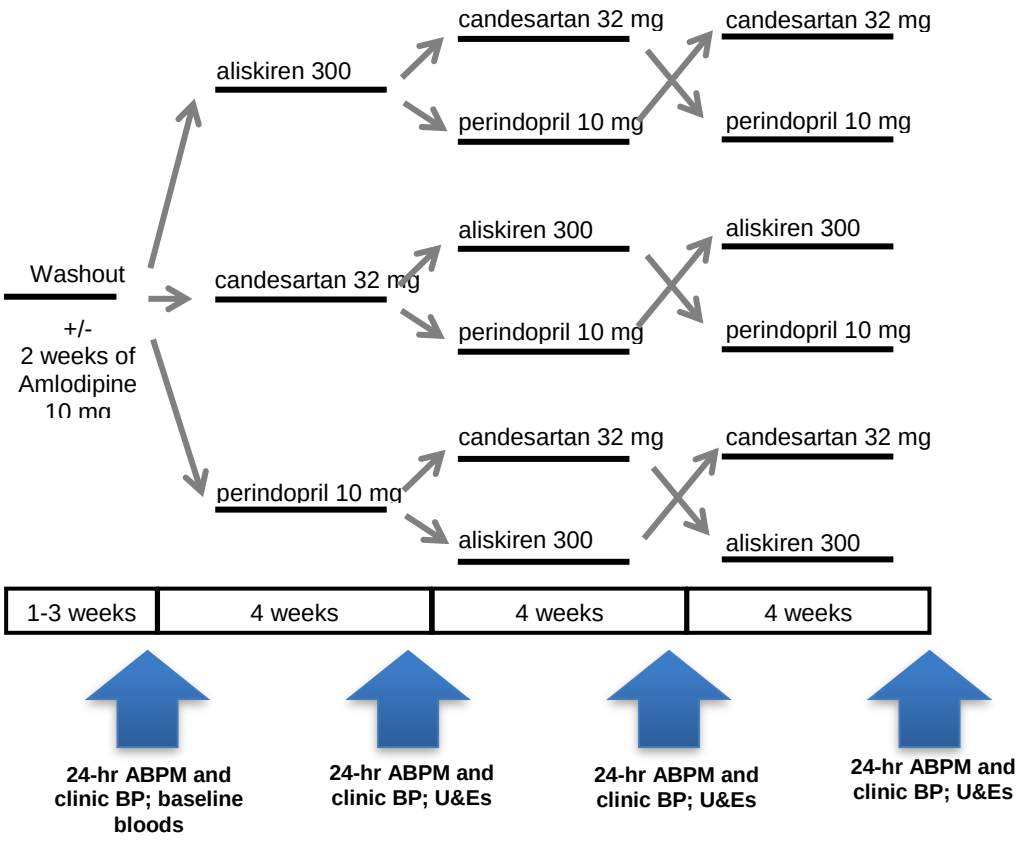
Upon completion of this evaluation, participants were then allocated in random order (stratified according to amlodipine usage or not), to 4 weeks of treatment with a renin inhibitor (Aliskiren 300 mg daily), an angiotensin receptor blocker (Candesartan 32 mg daily) and an angiotensin converting enzyme inhibitor (Perindopril 10 mg daily) (see Figure 1 overleaf). Randomisation was fixed, balanced and organised through use of sealed envelopes.

The design of this study is similar to a prospective randomized open therapy blinded end-points (PROBE) study. The primary end-point was the change from baseline in mean 24-hour systolic BP with each drug treatment. As ambulatory BP monitoring is fully automated, this is equivalent to an evaluator being blinded to treatment allocation.

At the end of each 4-week treatment period clinic BP, 24-hour ambulatory BP and serum urea, electrolytes and creatinine were measured. Adverse events and concomitant disease activity were evaluated. Details of all concomitant treatments were recorded. Participants were instructed to notify the study team regarding new medications taken after the start of the clinical trial.

At study end, participants were prescribed the medication(s) resulting in the best BP control during the study, without resulting in any clinically relevant side effects or adverse events. A letter summarizing their participation in the study was then sent to the participant’s GP.

Figure 1 **Schematic diagram demonstrating study design**



Selection of Study Population

Patients with mild to moderate essential arterial hypertension attending Beaumont Hospital cardiovascular outpatient clinics were invited to participate in the study. There was no upper age limit set for the study as the prevalence of hypertension increases with age.

Inclusion Criteria

Patients fulfilling the following criteria were eligible for inclusion in the study:

- Male or female outpatients
- Age greater than 18 years.
- Written informed consent provided.
- Patients with essential hypertension who are either antihypertensive treatment naïve, or taking a maximum of two antihypertensive agents – alpha-blockers already prescribed for indications other than hypertension, at a dose which will not change during the study, may be continued.
- Baseline mean 24-hour systolic pressure > 130 mm Hg, and < 160 mm Hg, either on no treatment or on amlodipine 10 mg daily.

Exclusion Criteria

Patients fulfilling the following criteria were excluded from participation in the study:

- Age less than 18 years old.
- Pregnancy, women who are breast feeding, or with childbearing potential without using a medically accepted method of contraception.

- Presence of any significant acute or chronic illness. In particular participants must not have a history of an acute cardiovascular morbid event within the last 3 months. They must not have suffered with malignant hypertension or congestive heart failure. They must not have a terminal illness.
- Significant baseline electrolyte, serum creatinine or creatinine clearance abnormalities (potassium > 5.5 mmol/l, or serum creatinine > 150 micromol/l, or creatinine clearance < 30mls/minute as assessed by Cockcroft's formula)
- Men = (140 – age) X weight (kg) / (0.814 X creatinine level (43icromole/l)
- Women = 0.85 X [(140 – age) X weight (kg) / (0.814 X creatinine level (micromole/l)]
- Known secondary hypertension
- Requirement for any specific antihypertensive drug therapy.
- Patients already taking three or more antihypertensive agents – alpha-blockers, when prescribed for indications other than hypertension, are not counted as an antihypertensive agent in this regard.
- Patients unable to discontinue current antihypertensive therapy – alpha-blockers already prescribed for indication other than hypertension, at a dose which will not change during the study, may be continued.
- Contra-indications to any of the study drugs.
- Participation in any other studies involving investigational or marketed products within one month prior to entry into this study or concomitantly with this study.
- Participants that are unlikely to comply well with study treatments or with the scheduled visits.
- Participants with a history of alcohol or drug abuse, psychosis, antagonistic personality, or any emotional or intellectual problems that are likely to invalidate informed consent, or limit the ability of the subject to comply with the protocol requirements

Withdrawal Criteria

Patients were free to withdraw from the study at any time without giving a reason. Patients were advised that if they requested to withdraw from the study, at any time during the trial, then this would have no negative consequences on further care

Study treatments were to be permanently discontinued for a participant for any of the following reasons;

- Onset of an adverse event which presented a risk to the patient, or which required a prescription of a treatment which is prohibited by the protocol
- Renal function impairment with a decrease in creatinine clearance by >30% as assessed by Cockcroft's formula
    - Men = (140 - age) X weight (kg) / (0.814 X creatinine level (micromol/l)
    - Women = 0.85 X [(140 - age) X weight (kg) / (0.814 X creatinine level (micromol/l)]
- Increased potassium level > 5.5 mmol/l, confirmed in a repeated sample assayed within 7 days.
- Pregnancy
- Worsening of hypertension, defined as mean 24-hour ambulatory systolic BP > 160 mmHg.
- Non-medical reason (patient's personal decision to stop treatment).

Method of Assigning Patients to Treatment Groups

Randomisation was fixed, balanced and organised through use of sealed envelopes. All participants were allocated, in random order (stratified according to amlodipine usage or not), to 4 weeks of treatment with a renin inhibitor (aliskiren 300 mg daily), an angiotensin receptor blocker (candesartan 32 mg daily) and an angiotensin converting enzyme inhibitor (perindopril 8 mg daily). See Figure 1.

Treatments Administered

All study drugs were supplied as commercially available tablets (Perindopril, Coversyl®: Servier; Candesartan, Atacand®: Astra Zeneca; Aliskiren, Rasilez®: Novartis) and were stored in a secured area with restricted access at room temperature. Temperature monitoring logs were undertaken daily. The dosages of the three RAS blockers and the calcium channel antagonist were the maximum licensed maintenance dosages recommended for treatment of uncomplicated hypertension. Study treatments were dispensed to participants in accordance with the study protocol, and in accordance with the individual randomization schedule.

The investigational product for each participant was supplied in a standard commercial medication box, comprising medication sufficient for 4 weeks of treatment and an additional one week supply. The medication box was labelled with a unique identifier, which corresponded to the patient's randomization number. The medication box required storage below 25^{o}C and the medication had to be protected from light and moisture.

The drugs used are all licensed for use in treatment of hypertension, and the doses given were all at their individual maximum licensed doses. These doses were chosen to avoid a potentially incomplete blood pressure response due to inadequate dosing with any one drug. The assumption was made a priori that there was no carry-over effect between treatment periods.

Treatment Adherence

Throughout the trial, patients were asked to take all study medication, once daily, 30 minutes prior to eating breakfast. The numbers of capsules/tablets dispensed at each visit, and the numbers returned at each visit were counted and recorded.

Efficacy and Safety Variables

Primary Efficacy End-Point

- The change from baseline in 24-hour systolic BP with each drug treatment.

Secondary Efficacy End-Points

- The change from baseline in 24-hour diastolic BP
- The change from baseline in daytime systolic BP
- The change from baseline in daytime diastolic BP
- The change from baseline in night-time systolic BP
- The change from baseline in night-time diastolic BP
- The proportion of participants with controlled BP, defined as mean 24-hour systolic BP < 130mmHg and mean 24-hour diastolic BP < 80mmHg
- The change from baseline in clinic systolic BP
- The change from baseline in clinic diastolic BP

Safety Endpoints

- Adverse events
- Assessment of concomitant diseases
- Clinic BP and HR
- Ambulatory BP monitoring
- Urea, electrolytes and creatinine estimation

Assessment of Efficacy and Safety Variables

Table 1 shows the schedule of assessments and procedures.

Medical history and Examination

On the initial screening visit a full medical history and examination was performed. Current diagnoses, past medical history, current medications, life-style assessment including diet, smoking history and family history were evaluated. Alpha-blockers already prescribed for indications other than hypertension (for example, in the treatment of benign prostatic hypertrophy, BPH), at a dose that would not change during the study were continued.

The participants' height, weight and waist circumference were measured with participants standing without shoes and in basic indoor clothing. Weight was measured to the nearest 0.1kg using Seca scales (Seca, Hamburg, Germany) that were calibrated regularly. Height was measured to the nearest 5mm on a Seca height gauge (Seca, Hamburg, Germany). A full cardiovascular examination was performed.

Electrocardiography (ECG)

A standard 12 lead ECG was performed at screening, and reviewed for abnormalities, and also on any occasion during the study where clinically indicated.

Phlebotomy

At baseline and off all antihypertensive medications (except alpha-blockers), erect and supine phlebotomy was performed with blood samples taken after 10 minutes in the erect position and after 20 minutes in the supine position (Sarstedt Monovette blood collection system).

At the baseline visit, phlebotomy was performed to allow the following:
• Measurement of baseline renal function - serum urea, electrolyte, creatinine and glomerular filtration rate
• Measurement of serum cholesterol and triglycerides, serum glucose
• Measurement of plasma renin activity, both upright and repeated after 20 minutes supine
• Immediate spinning down of blood samples and storage of plasma aliquots for future measurements
• DNA extraction for REN-5312C/T genotyping and storage of DNA for future genetic studies (Beaumont Hospital Medical Research Ethics Committee reference 08/60, date of approval 7th August 2008).

Table 1 Schedule of assessments and procedures

	Week -1 or -3 Screening	Week -2		Week 0 Baseline		Week 4		Week 8		Week 12	
	V1	Extra visits *		V2	V3	V4	V5	V6	V7	V8	V9
Informed consent	X										
Medical history	X										
Physical examination	X										
ECG	X										
Inclusion/exclusion criteria			X		X						
Urea, electrolytes and creatinine estimation				X		X		X		X	
Erect and supine PRA				X							
Erect and supine Serum storage				X							
Blood storage for DNA extraction and storage and REN-5312C/T genotyping				X							
Clinic BP measurement	X	X		X		X		X		X	
Placement of ABPM monitor		X		X		X		X		X	
Removal of ABPM monitor					X		X		X		X
Adverse events			X		X		X		X		X
Assessment of concomitant diseases and medications	X		X		X		X		X		X
Dispensing of study medication			X		X		X		X		
Assessment of compliance with study medication					X		X		X		X

* Two extra visits will be required for those participants whose off treatment mean 24-hour systolic pressure is > 160 mm Hg and < 175 mm Hg. These participants will be prescribed amlodipine 10 mg daily, and a second baseline visit scheduled for 2 weeks later.

Visit windows will be at the designated timing plus/minus one week. Hence the minimum and maximum duration of treatment on each RAS blocker will be 21 and 35 days respectively.

In total 60 mLs were drawn at the baseline visit, 15 mLs in the erect position, and 45 mLs in the supine position. On subsequent study visits, blood sampling occurred in the sitting position and was performed for renal function estimation only with a volume of up to 7.5mL taken.

Clinic Blood Pressure Measurement

Seated clinic BP measurement and heart rate (HR) were measured from the right arm using a regularly calibrated validated automated sphygmomanometer (Omron HEM-705CP). After at least 5 minutes in the sitting position, three measurements were taken at one-minute intervals. Seated BP and HR were taken as the average of the second and third readings.

Ambulatory Blood Pressure Measurement

Ambulatory Blood Pressure Measurements (ABPM) using the oscillometric method were obtained every half-hour throughout the 24-hour period using SpaceLabs 90207 monitors (SpaceLabs Medical Inc. Issaqua, West Virginia, USA). Monitoring commenced between 07.00 and 14.00 hours on each occasion. Both study medication and concomitant medication were taken at the usual timing on the days where ABPM was performed. The ABPM reading was regarded as satisfactory if there were at least 14 daytime readings and 8 night-time readings. These were deemed the minimum acceptable number of readings to ensure the ABPM result was eligible for inclusion in study analysis.

The first and subsequent study medications were not commenced until the baseline and each ABPM thereafter was judged to have been satisfactory. In instances where the minimum number of ABPM measurements were not achieved, a second ABPM was placed within 24-72 hours. Where a second ABPM needed to be performed to satisfy the requirements of the analysis of data obtained, the medication period was extended by 24-72 hours. This was to ensure each end-of-treatment ABPM was a valid assessment of response to antihypertensive treatment. Mean 24-hour, daytime (mean of all readings obtained between 0900 and 2100), night-time systolic and diastolic blood pressures were calculated from the ABPMs.

Immediate Blood Assays

Serum urea, electrolytes and creatinine were quantified at baseline and at the end of each treatment period. Cholesterol, triglycerides and glucose were also quantified as they are known additional risk factors in cardiovascular disease. These biochemical blood assays were performed by Beaumont Hospital Clinical Laboratory.

Blood Sample Storage, DNA Extraction and Future Assays

At the baseline visit only, additional erect and supine blood samples were taken. These blood samples were immediately centrifuged at 3500 rpm for 15 minutes, the plasma was pipetted off, and up to four 1 ml plasma aliquots were stored at -80OC in Cryovials (Cruinn Diagnostics).

One 1 ml aliquot was assayed to determine PRA within one month as considerable variations in PRA have been observed when samples were stored at 2-8^{o}C or when stored frozen at -20^{O}C for prolonged periods when frozen (Locsei et al. 2009; Sealey et al, 1991). PRA was measured by radioimmunoassay (GammaCoat Plasma Renin Activity 125I RIA Kit, CA-1533; DiaSorin, Stillwater, MN) at St James' Hospital, Dublin 8. Prior to assay the frozen samples were rapidly defrosted to room temperature. Plasma renin activity determination involved an initial incubation of plasma to generate angiotensin I (up to 18 hours). Phenylmethylsulfonyl fluoride (PMSF) was added to the samples to prevent enzymatic conversion of angiotensin I to angiotensin II (GammaCoat Plasma Renin Activity 125I RIA Kit, Diasorin). Maleate generation buffer was also added to the samples to maximize the generation of angiotensin I from angiotensinogen. Angiotensin I generation was followed by quantitation of angiotensin I by a three-hour radioimmunoassay incubation (Rabbit anti-Angiotensin I coated tubes). PRA activity was expressed as ng/mL/hour of generated Angiotensin I.

The remainder of the plasma aliquots remain in storage so as to allow future measurements of

bioactive molecules. The specific assays that will be performed are currently undetermined.

At the baseline visit only, whole blood samples were preserved in EDTA and immediately frozen at -80^{O}C to allow later DNA extraction for renin SNP genotyping, and storage of DNA for future genetic studies.

Blood DNA Extraction

The following materials were used for the purpose of leukocyte and DNA extraction:

- Specified Laboratory Reagent (SLR) Solution (TRIS 2M pH 7.6, MgCl2 1M, NaCl 3M and distilled water)
- 50mL Sigma-Aldrich Greiner tubes
- Centrifuge, vortex and Gilsen pipette and pipette tips
- Gilsen “Macroman” pipette and 10mL, 20mL large plastic pipette tubes
- Phosphate-buffered saline (PBS)(Sigma-Aldrich), ethanol (96-100%) (Sigma-Aldrich), Proteinase K 50µg/mL (Sigma-Aldrich)
- Water bath heated to 37ºC
- Waste Container
- QIAamp DNA Blood Mini Kit (Qiagen), QIAcube machine (Qiagen)

Frozen blood was thawed in the heated water bath and lysed to obtain white cells. The QIAmp DNA Blood Mini Kit (Qiagen) and Qiagen QIACube were used to extract DNA from the white cells.

Extraction of Leukocytes: The 7.5mL EDTA bottles (Sarstedt Monovette) were removed from the -80ºC freezer and heated to 37oC in a water-bath until thawed. SLR was freshly prepared in sterile graduate cylinders using sterile distilled water and stored at +4°C. The thawed samples were centrifuged in 50mL volume Sarstedt tubes, the plasma aspirated and discarded without disturbing the buffy coat. Mixing of the remaining red blood cells and leukocytes occurred and the volume was made up to 50mL with SLR, vortexed to resuspend and centrifuged at 3500 rpm for fifteen minutes. The remaining cells underwent lysis as a result of mixing with SLR to obtain a cellular pellet. The supernatant was removed, and further SLR was added to the Sarstedt tubes with two further repeat resuspensions and centrifugations at 3500 rpm for fifteen minutes. A cellular pellet was obtained which was then frozen at -80°C.

DNA Purification from Leukocytes: The cellular pellet was defrosted in a water bath at 50ºC for one minute with lysis of the leuckocytes in 1x phosphate buffered saline (PBS) (137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and pH of 7.4) and 50µg/mL proteinase K.

The Qiagen DNA Mini Kit buffers (Qiagen) and proteinase K (Sigma-Aldrich) were prepared in accordance with the kit protocol. Leukocytes were suspended in a mixture of PBS (Sigma-Aldrich) and 50µg/mL proteinase K (Sigma-Aldrich), with the kit Buffer AL (Qiagen) added to complete the lysis procedure. The samples were incubated, mixed with high concentration 96-100% ethanol, centrifuged and the resulting mixture transferred to the spin column. The kit buffers were added in sequence, samples centrifuged with each sequential buffer addition and the isolated DNA eluted. The procedure followed was exactly as per manufacturer’s protocol (Qiagen). DNA was then stored at –20°C in Buffer AE (Qiagen) to avoid acid hydrolysis.

Determination of DNA concentration obtained: DNA concentrations in the samples prior to freezing were determined using the Nanodrop ® 8000 UV-Vis Spectrophotometer. The spectrophotometer was blanked using 100µL of H2O and samples loaded with 8 x 1µL samples loaded at a time. Nucleic acid concentration and purity were measured. The A260/A280 ratio was used to evaluate sample purity. Nucleic acids and proteins have absorbance maxima at 260 and 280 nm, respectively. The A260/A280 ratio is a ratio of spectrophotometric absorbance of the sample at 260 nm to that of 280 nm, and is used to assess purity of nucleic acid samples in DNA extraction. A ratio of approximately 1.8 is generally accepted as “pure” for DNA (Glasel 1995; Murray & Rajeevan 2013).

DNA Plating and Genotyping:

DNA was pipetted carefully onto a sterile 96-well plate (10 µL at 10ng/ µLper SNP) (Greiner, Sigma-Aldrich). Random duplicates were incorporated with new sample IDs along with two blank wells as controls. The plates were left to dry overnight. Adhesive film was placed on top prior to packaging and preparation for transport. The plates were sent by registered airfreight to K Biosciences, Herts. Genotyping was performed by Kbiosciences using modified TaqMan assays (http://www.kbioscience.co.uk). Genotype calling was performed using Kluster software. Samples were excluded from the analysis with a genotyping call rate of <95%. All declared and hidden blanks were correctly called, and all hidden sample duplications were consistent.

Data Handling and Record Keeping

All data generated by the study remains confidential and no report or results contain any information that would allow an individual participant in the study to be identified. Paper clinical record forms were used in this study. Data was also stored electronically using EXCEL (Microsoft EXCEL 97, Microsoft Corporation, Redmond, WA, USA) database. Electronically stored data was identified by a unique registration number. All information relevant to the study is to be stored for at least 15 years after the end of the study.

Sample Size and Study Power

Assuming a standard deviation of 6 mmHg for the change in baseline to end-of-treatment 24-hour systolic BP, a significance level of 0.05, 90 evaluable patients, and a renin -5312C/T minor allele frequency of 30%, this study had 80% power to detect 4 mm Hg differences in the primary end-point (change from baseline to end-of-treatment mean 24-hour systolic BP) with treatment with a renin inhibitor, an angiotensin receptor blocker, and an angiotensin converting enzyme inhibitor, between REN-5312 CC homozygotes versus CT heterozygotes versus TT homozygotes.

Statistical Analyses

Phenotypic data were expressed as mean ± standard deviation, as median [interquartile range], or as numbers (percentages). PRA followed a lognormal distribution and therefore was log-transformed before statistical analysis.

Departure from Hardy-Weinberg equilibrium was tested by Chi-squared tests. Genotypic analyses involved comparisons of phenotypic characteristics of REN-5312 CC homozygotes, CT heterozygotes and TT homozygotes.

The principal hypothesis tested within this clinical trial was whether REN-5312C/T genotype, alone or in combination with PRA, provides improved prediction of BP lowering responses to RAS blockade, additional to that provided by drug type, age, and baseline BP. The primary endpoint was the change from baseline in mean 24-hour systolic blood pressure with each drug therapy. Secondary endpoints included the changes from baseline in daytime systolic, night-time systolic, 24-hour diastolic, daytime diastolic and night-time diastolic blood pressures with the three drug therapies.

Generalised linear modelling (stepwise regression models with backward elimination) was used to test for independent predictors of BP lowering responses. The stepwise regression was performed by iteratively removing non-significant ($p<0.05$) variables from the model until only variables significantly associated with BP lowering responses remained. In order to test for additional prediction of BP lowering responses by REN-5312C/T genotype, alone or in combination with PRA, four linear regression models were tested for each endpoint;

- Model 1 included age, gender, drug treatment, and baseline BP as covariates.

- Model 2 included age, gender, drug treatment, baseline BP, and log supine PRA as covariates.
- Model 3 included age, gender, drug treatment, baseline BP, REN-5312C/T genotype, REN-5312C/T genotype*drug treatment interaction and REN-5312C/T genotype*baseline BP interaction as covariates.
- Model 4 included age, gender, drug treatment, baseline BP, log supine PRA, REN-5312C/T genotype, REN-5312C/T genotype*drug treatment interaction, REN-5312C/T genotype*baseline BP interaction and REN-5312C/T genotype*log supine PRA interaction as covariates.

Of note, repeated measurements were taken after four weeks of treatment on the assumption that there were no residual carryover effects from the previous treatment after that duration. If it is believed a priori that carryover does not exist, then it is thought appropriate to disregard the possibility of its existence in the statistical model employed {Senn:2003tn}- .

Analyses were performed using DataDesk® statistical software package version 7.0.2 (Data Description, Inc., Ithaca, NY 14852-4555, USA). All statistical tests were 2-sided. For all analyses $P<0.05$ was considered statistically significant.

9 STUDY POPULATION

One hundred and twenty-four subjects were screened for eligibility for this study. Ninety-eight subjects met entry criteria and were randomised to the study. Figure 2 shows the number of subjects contributing to the population for this study. Table 2 details the reasons for exclusion of the 26 participants who were not randomised.

Baseline Characteristics According to Gender

Of the 98 participants successfully enrolled in the study, female and male study participants were of a similar age and did not differ for smoking habit. Baseline 24-hour systolic and diastolic blood pressures and PRA were also similar for female and male participants.

Alcohol intake was significantly higher amongst male participants. There were statistically significant differences between female and male participants in weight, waist circumference, creatinine, and HDL-cholesterol levels (with respective p-values all <0.001). Fasting glucose was statistically different between females and males with a p-value of 0.02.

These data are illustrated in table 3.

Baseline Characteristics according to REN-5312C/T Genotype

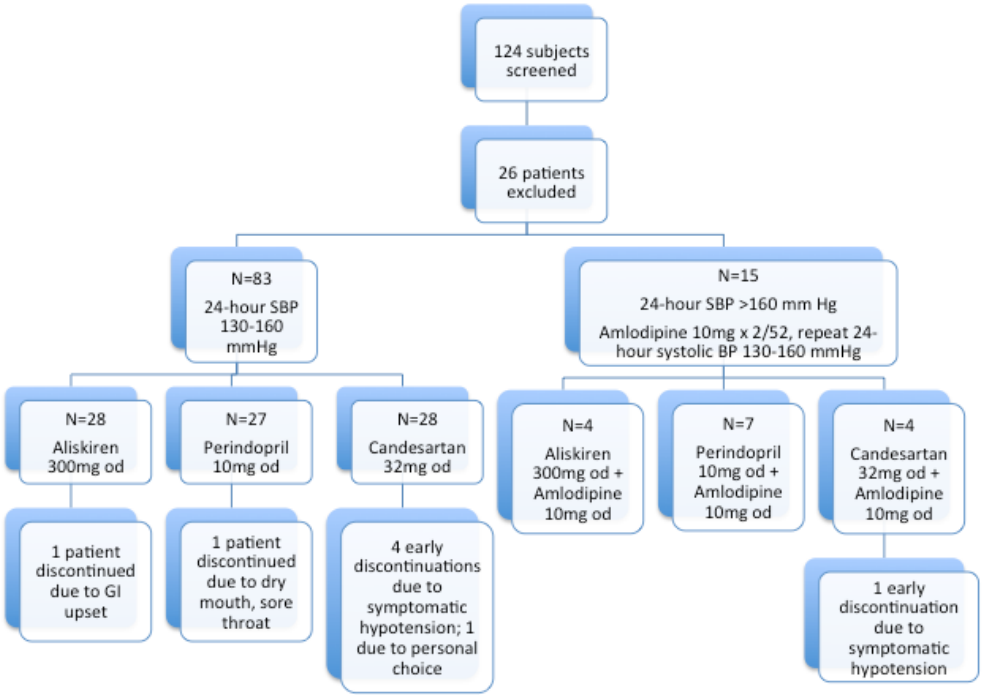
With a minor allele frequency of 0.18, the population was found to be in Hardy-Weinberg Equilibrium, with the HWE equation, $p^2 + 2pq + q^2 = 1$, fulfilled:

$$0.67 + 0.29 + 0.03 = 0.99.$$

Most baseline characteristics did not differ between the three REN-5312C/T genotypes. Age, body mass index, waist circumference, smoking habit, alcohol intake, exercise habit, blood pressure, lipid profile and glycaemia were similar. These data are presented in the table overleaf (Table 4).

However PRA and serum potassium did differ significantly according to REN-5312C/T genotype. These data are illustrated in table 4 and figure 3.

Figure 2 - Study population.



N, number; mmHg, millimetres of Mercury; od, once daily; GI, gastrointestinal.

Table 2 - Reasons for 26 subjects being excluded from participation in the study.

Number	Exclusion Reason
12	Baseline 24-hour SBP after one-week washout period of <130 mmHg
4	24-hour systolic blood pressure <130 mmHg after treatment with amlodipine 10mg as per protocol
3	Patient change of mind
1	ECG revealed widespread ischaemic changes, urgent Cardiology referral with subsequent percutaneous intervention undertaken
1	Hypokalaemia on screening bloods, investigated for secondary hypertension
1	Deranged thyroid function with a palpable goitre
1	Left bundle branch block of unknown cause
1	Elevated creatinine of 150 µmol/L
1	Previous amlodipine intolerance in a patient in whom treatment with the drug was indicated (washout 24-hour SBP >160 mmHg)
1	Poor understanding of the trial procedures

mmHg, millimetres of Mercury; µmol/L, micromoles per litre

Table 3 – Population Characteristics by Gender.

Variable		Male	Female	p-value
		n=63	n=35	
Age, years		55.1(9.6)	51.9(9.2)	0.106
Weight, kilograms (kg)		93.1(14.7)	75.6(15.3)	<0.001*
Body Mass Index (kg/m2)		29.9(4.4)	28.3(4.7)	0.09
Waist, centimetres (cm)		107.4(10.9)	95.3(11.6)	<0.001*
Smoking	Current smoker	13(21%)	3(8.5%)	0.904
	Ex-smoker	23(36.5%)	9(25.7%)	
	Never-smoker	27(42.8%)	22(62.8%)	
Alcohol intake, units per week		14.3(13.7)	6.6(6.9)	0.002*
Regular exercise	Yes	32(50.8%)	24(68.5%)	0.09
	No	31(49.2%)	11(31.4%)	
Baseline 24-hour Systolic Blood Pressure (mmHg)		142.5(9.0)	141.7(6.9)	0.32
Baseline 24-hour Diastolic Blood Pressure (mmHg)		86.3(6.4)	85.1(6.8)	0.35
Total cholesterol	(mmol/L)	4.9(1.1)	5.3(1.2)	0.11
Triglycerides	(mmol/L)	2.0(1.7)	1.5(1.0)	0.09
HDL cholesterol	(mmol/L)	1.3(0.3)	1.6(0.5)	<0.001*
LDL cholesterol	(mmol/L)	2.8(0.9)	3.0(1.0)	0.26
Fasting glucose	(mmol/L)	5.6(1.0)	5.1(1.2)	0.02*
Serum sodium	(mmol/L)	139.5(2.3)	139.7(2.1)	0.59
Serum potassium	(mmol/L)	4.0(0.3)	4.0(0.4)	0.89
Serum chloride	(mmol/L)	104.9(2.5)	105.5(1.5)	0.22
Creatinine	(micromol/L)	80.1(11.5)	63.8(10.8)	<0.001*
GFR	(mL/kg/hr)	88.3(15.4)	90.7(26.7)	0.57
Baseline PRA, upright	(ng/mL/hr)	1.6(1.4)	1.5(1.0)	0.54
Baseline PRA, supine	(ng/mL/hr)	1.3(1.3)	1.3(1.3)	0.99
Log PRA, upright		0.1(0.3)	0.1(0.3)	0.59
Log PRA, supine		0.0(0.4)	0.0(0.4)	0.8
REN-5312C/T	CC-homozygote	42(66.6%)	25(71.4%)	0.85
	CT-heterozygote	19(30.1%)	8(22.8%)	
	TT-homozygote	2(3.1%)	2(5.7%)	

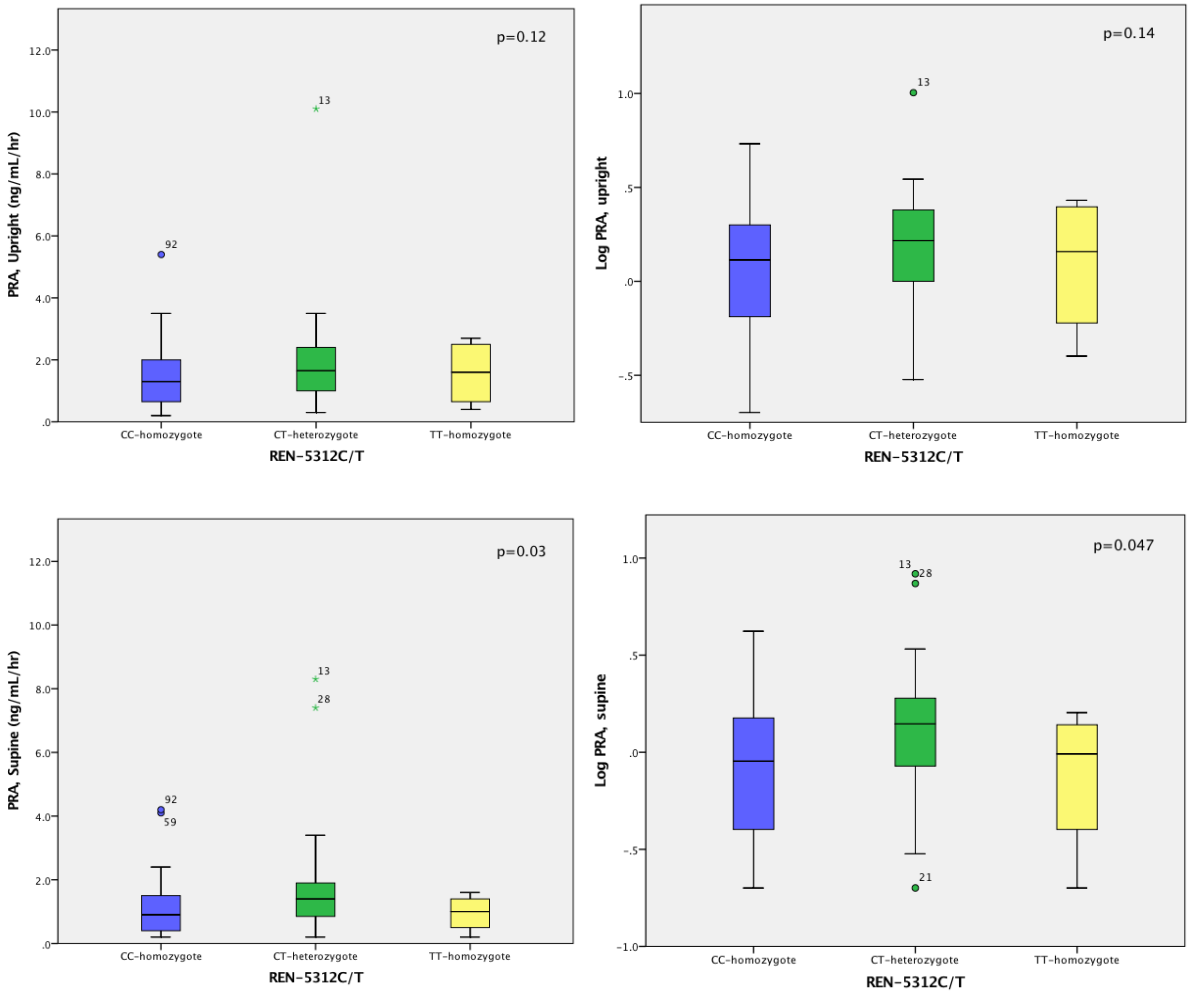
Data expressed as mean and standard deviation, mean (SD), for continuous variables; count and percentage for categorical variables. Analysis of Variance and Chi-squared test used to determine significance values. mm Hg, millimetres of Mercury; PRA, plasma renin activity; kg, kilograms; ng/mL/hr, nanograms per milliliter per hour of Angiotensin I generation; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol

Table 4 – Population characteristics by REN-5312C/T genotype.

Variable	Subgroup	CC; n=67	CT; n=27	TT; n=4	p-value
Age, years		53.1(8.9)	56.6(10.4)	51(13.2)	0.22
Gender	Male	42	19	2	0.66
	Female	25	8	2	
Weight, kilograms (kg)		87.4(17.6)	85.4(16.5)	88.4(14.2)	0.86
Body Mass Index, BMI	kg/m2	29.4(4.7)	29.2(4.5)	29.3(2.4)	0.97
Waist, centimetres (cm)		103.1(13)	102.4(12)	107.5(9.9)	0.75
Smoking	Current	10(14.9%)	5(18.5%)	1(25%)	0.96
	Ex-smoker	21(31.3%)	9(33.3%)	2(50%)	
	Never smoke	35(52.2%)	13(48.1%)	1(25%)	
Alcohol intake	Units per we	11.1(12.1)	12.8(13.2)	10.5(9)	0.83
Added dietary salt	Yes	25(37.3%)	6(22.2%)	1(25%)	0.36
	No	42(62.6%)	21(77.7%)	3(75%)	
Regular exercise	Yes	38(56.7%)	15(55.5%)	3(75%)	0.76
	No	29(43.2%)	12(44.4%)	1(25%)	
Diabetes, known	Yes	5(7.4%)	2(7.4%)	0(0%)	0.86
	No	62(92.5%)	25(92.5%)	4(100%)	
Baseline 24-hour SBP (mmHg)		142.4(7.7)	144.6(9.5)	138(9.4)	0.26
Baseline 24-hour DBP (mmHg)		85.8(6.2)	86.1(7.5)	85.5(6.9)	0.97
Total cholesterol	(mmol/L)	5(1.2)	5.1(1.1)	5.3(0.8)	0.87
Triglycerides	(mmol/L)	2(1.8)	1.4(0.6)	1.7(0.4)	0.31
HDL cholesterol	(mmol/L)	1.4(0.4)	1.4(0.4)	1.4(0.3)	0.9
LDL cholesterol	(mmol/L)	2.8(1)	3(0.9)	3.1(0.7)	0.59
Fasting glucose	(mmol/L)	5.5(1.2)	5.4(0.9)	4.7(0.9)	0.34
Serum sodium	(mmol/L)	139.6(2.4)	139.4(2)	140(0)	0.87
Serum potassium	(mmol/L)	4(0.3)	4(0.3)	4.4(0.2)	0.02*
Creatinine	(micromol/L)	74.9(14.9)	72.9(10.3)	73.3(14.4)	0.8
GFR	(mL/kg/hr)	89.6(22.6)	88.2(14)	86.5(9.5)	0.92
Baseline PRA, upright	(ng/mL/hr)	1.4(0.9)	2(1.9)	1.6(1.1)	0.12
Baseline PRA, supine	(ng/mL/hr)	1.1(0.8)	1.8(1.9)	1(0.6)	0.03*
Log PRA, upright		0(0.3)	0.2(0.3)	0.1(0.4)	0.14
Log PRA, supine		−0.1(0.3)	0.1(0.4)	−0.1(0.4)	0.04*

Data expressed as mean and standard deviation, mean (SD), for continuous variables; count and percentage, %, for categorical variables. N, number; mmHg, millimeters of Mercury; mmol/L, millimoles per litre; mL/kg/hr, milliliter per kilogram per hour; ng/mL/hr, nanograms per milliliter per hour.

Figure 3 – Boxplots illustrating relationship between PRA, upright and supine, and renin -5312C/T genotype.



P-values obtained by one-way ANOVA with Bonferroni adjustment.

10 STUDY EFFICACY RESULTS

Independent Predictors of BP Lowering Responses

The principal hypothesis tested within this clinical trial was whether REN-5312C/T genotype, alone or in combination with PRA, provided improved prediction of BP lowering responses to RAS blockade. The following generalised linear models (stepwise regression models with backward elimination) were used to test for independent predictors of BP lowering responses. Four linear regression models were tested for each endpoint;

- Model 1 included age, gender, drug treatment, and baseline BP as covariates.
- Model 2 included age, gender, drug treatment, baseline BP, and log supine PRA as covariates.
- Model 3 included age, gender, drug treatment, baseline BP, REN-5312C/T genotype, REN-5312C/T genotype*drug treatment interaction and REN-5312C/T genotype*baseline BP interaction as covariates.
- Model 4 included age, gender, drug treatment, baseline BP, log supine PRA, REN-5312C/T genotype, REN-5312C/T genotype*drug treatment interaction, REN-5312C/T genotype*baseline BP interaction and REN-5312C/T genotype*log supine PRA interaction as covariates.

The results from these models for all 6 endpoints (24-hour, daytime, night-time, systolic and diastolic pressures) are presented in the tables 5 - 8.

It is clear from these tables that blood pressure lowering responses were greater;

- With candesartan treatment compared with aliskiren or perindopril treatments;
- In females compared to males;
- In participants with higher blood pressures;
- In participants with higher PRA levels;
- In REN-5312T allele carriers who also had higher BP levels and/or lower PRA levels.

The associations with REN-5312C/T genotype are further illustrated in Figures 4-8.

Table 5 - Independent Predictors of Blood Pressure Lowering Responses - Model 1: including Age, Gender, Treatment and Baseline BP as covariates. BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; N/S, non-significant**.**

		24 Hour SBP		Daytime SBP		Night-time SBP		24 Hour DBP		Daytime DBP		Night-time DBP	
Factor	Level	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value
Intercept		69.3(12.0)	≤0.0001	75.3(12.3)	≤0.0001	44.7(10.2)	≤0.0001	44.1(7.7)	≤0.0001	35.9(5.9)	≤0.0001	27.9(5.7)	≤0.0001
Age			N/S		N/S		N/S		N/S		N/S		N/S
Gender (female)		-5.95(1.45)	≤0.0001	-5.95(1.56)	0.0002	-6.28(1.71)	0.0003	-3.63(0.95)	0.0002	-3.02(1.02)	0.0035	-4.57(1.18)	≤0.0001
Treatment			0.0009		0.0057		0.0496		0.0004		0.0038		N/S
	Aliskiren	2.14(0.96)	0.0277	2.54(1.03)	0.0145	1.11(1.15)	0.3346	1.43(0.62)	0.0227	1.73(0.69)	0.0124		
	Candesartan	-3.64(0.96)	0.0002	-3.16(1.03)	0.0024	-2.81(1.15)	0.0150	-2.47(0.62)	≤0.0001	-2.20(0.69)	0.0016		
	Perindopril	1.50(0.97)	0.1222	0.62(1.04)	0.5492	1.70(1.15)	0.1410	1.04(0.62)	0.0949	0.47(0.69)	0.4987		
Baseline BP		-0.58(0.08)	≤0.0001	-0.61(0.08)	≤0.0001	-0.44(0.08)	≤0.0001	-0.55(0.07)	≤0.0001	-0.49(0.06)	≤0.0001	-0.46(0.07)	≤0.0001
r squared value		0.22		0.21		0.16		0.23		0.22		0.16	

Table 6 - Independent Predictors of Blood Pressure Lowering Responses - Model 2: including age, gender, drug treatment, baseline BP, and log supine PRA as covariates. BP, blood pressure; PRA, plasma renin activity; SBP, systolic blood pressure; DBP, diastolic blood pressure; N/S, non-significant.

		24 Hour SBP		Daytime SBP		Night-time SBP		24 Hour DBP		Daytime DBP		Night-time DBP	
Factor	Level	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value
Intercept		69.2(11.8)	≤0.0001	75.9(12.0)	≤0.0001	44.0(10.1)	≤0.0001	43.6(7.3)	≤0.0001	43.9(7.5)	≤0.0001	35.2(7.0)	≤0.0001
Age			N/S		N/S		N/S	-0.16(0.05)	0.0014	-0.14(0.06)	0.0114	-0.13(0.06)	0.0349
Gender (female)		-6.08(1.42)	≤0.0001	-6.11(1.56)	≤0.0001	-6.22(1.69)	0.0002	-3.91(0.91)	≤0.0001	-3.66(1.00)	0.0003	-5.12(1.17)	≤0.0001
Treatment			0.0007		0.0046		0.0433		0.0002		0.0026		0.0479
	Aliskiren	2.09(0.95)	0.0280	2.49(1.01)	0.0145	1.07(1.13)	0.3447	1.37(0.59)	0.0213	1.65(0.66)	0.0125	0.74(0.77)	0.3343
	Candesartan	-3.66(0.95)	0.0001	-3.19(1.01)	0.0018	-2.84(1.14)	0.0131	-2.49(0.59)	≤0.0001	-2.20(0.66)	0.0009	-1.89(0.77)	0.0144
	Perindopril	1.57(0.95)	0.0997	0.70(1.02)	0.4934	1.77(1.14)	0.1234	1.12(0.59)	0.0611	0.55(0.66)	0.4082	1.15(0.77)	0.1377
Baseline BP		-0.59(0.08)	≤0.0001	-0.61(0.08)	≤0.0001	-0.43(0.08)	≤0.0001	-0.51(0.07)	≤0.0001	-0.49(0.07)	≤0.0001	-0.46(0.07)	≤0.0001
Ln PRA		-6.42(1.89)	0.0008	-7.07(2.02)	0.0005	-5.83(2.25)	0.0103	-6.57(1.23)	≤0.0001	-6.83(1.37)	≤0.0001	'-4.86(1.59)	0.0025
r squared value		0.25		0.25		0.18		0.31		0.29		0.22	

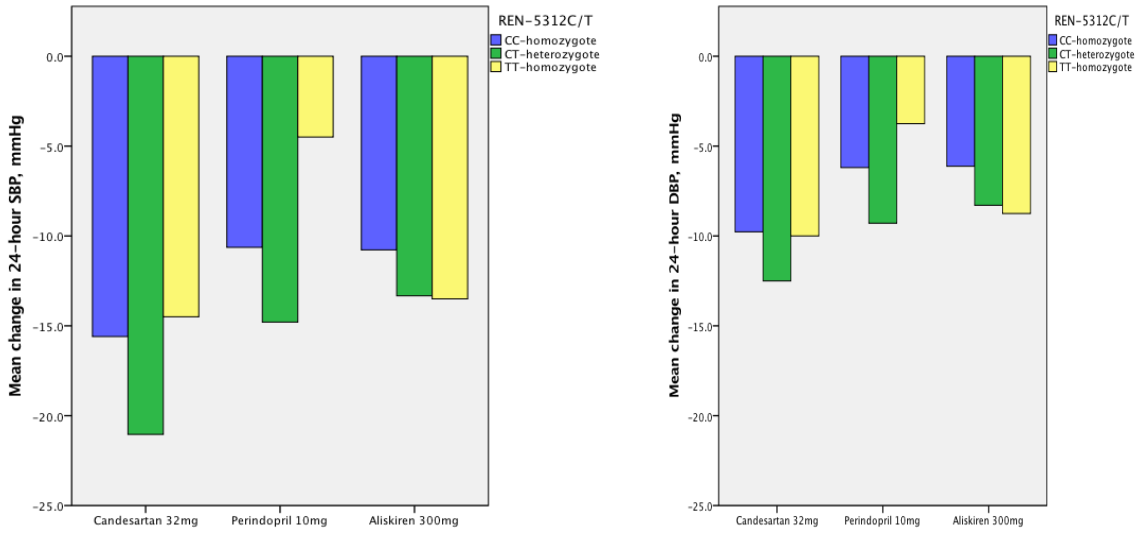
Table 3.1 - Independent Predictors of Blood Pressure Lowering Responses - Model 3: including age, gender, drug treatment, baseline BP, REN-5312C/T genotype, REN-5312C/T genotype*drug treatment interaction and REN-5312C/T genotype*baseline BP interaction as covariates. BP, blood pressure; PRA, plasma renin activity; SBP, systolic blood pressure; DBP, diastolic blood pressure; N/S, non-significant.

		24 Hour SBP		Daytime SBP		Night-time SBP		24 Hour DBP		Daytime DBP		Night-time DBP	
Factor	Level	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value
Intercept		110.3(20.2)	≤0.0001	116.9(22.0)	≤0.0001	99.3(36.2)	≤0.0001	44.1(7.7)	≤0.0001	35.9(5.9)	≤0.0001	27.9(5.7)	≤0.0001
Age			N/S		N/S		N/S		N/S		N/S		N/S
Gender (female)		-6.11(1.47)	≤0.0001	-5.70(1.59)	0.0004	-6.66(1.71)	0.0001	-3.63(0.95)	0.0002	-3.02(1.02)	0.0035	-4.57(1.18)	≤0.0001
Treatment			0.0009		0.0058		0.0500		0.0004		0.0038		N/S
	Aliskiren	2.11(0.95)	0.0278	2.51(1.03)	0.0150	1.05(1.13)	0.3545	1.43(0.62)	0.0227	1.73(0.69)	0.0124		
	Candesartan	-3.61(0.95)	0.0002	-3.14(1.03)	0.0024	-2.75(1.14)	0.0160	-2.47(0.62)	≤0.0001	-2.20(0.69)	0.0016		
	Perindopril	1.50(0.96)	0.1188	0.63(1.03)	0.5407	1.70(1.14)	0.1375	1.04(0.62)	0.0949	0.47(0.69)	0.4987		
Baseline BP		-0.88(0.14)	≤0.0001	-0.90(0.15)	≤0.0001	-0.85(0.27)	0.0021	-0.55(0.07)	≤0.0001	-0.49(0.06)	≤0.0001	-0.46(0.07)	≤0.0001
REN-5312C/T Genotype			0.0262		0.0446		0.0323		N/S		N/S		N/S
	TT	95.1(37.6)	0.0119	103.0(41.1)	0.0129	96.6(71.3)	0.1765						
	CT	-37.3(23.7)	0.1168	-57.0(25.8)	0.0277	-24.3(37.7)	0.5208						
	CC	-57.9(22.1)	0.0092	-45.9(23.6)	0.0532	-72.4(36.8)	0.0506						
REN-5312C/T*Baseline BP Interaction			0.0250		0.0452		0.0244		N/S		N/S		N/S
	TT	-0.68(0.27)	0.0123	-0.71(0.29)	0.0130	-0.73(0.54)	0.1807						
	CT	0.26(0.17)	0.1214	0.39(0.18)	0.0283	0.17(0.29)	0.5505						
	CC	0.42(0.27)	0.0080	0.33(0.16)	0.0453	0.56(0.28)	0.0480						
r squared value		0.24		0.23		0.19		0.23		0.22		0.16	

Table 3.2 - Independent Predictors of Blood Pressure Lowering Responses – Model 4: including age, gender, drug treatment, baseline BP, log supine PRA, REN-5312C/T genotype, REN-5312C/T genotype*drug treatment interaction, REN-5312C/T genotype*baseline BP interaction and REN-5312C/T genotype*log supine PRA interaction as covariates. BP, blood pressure; PRA, plasma renin activity; SBP, systolic blood pressure; DBP, diastolic blood pressure; N/S, non-significant.

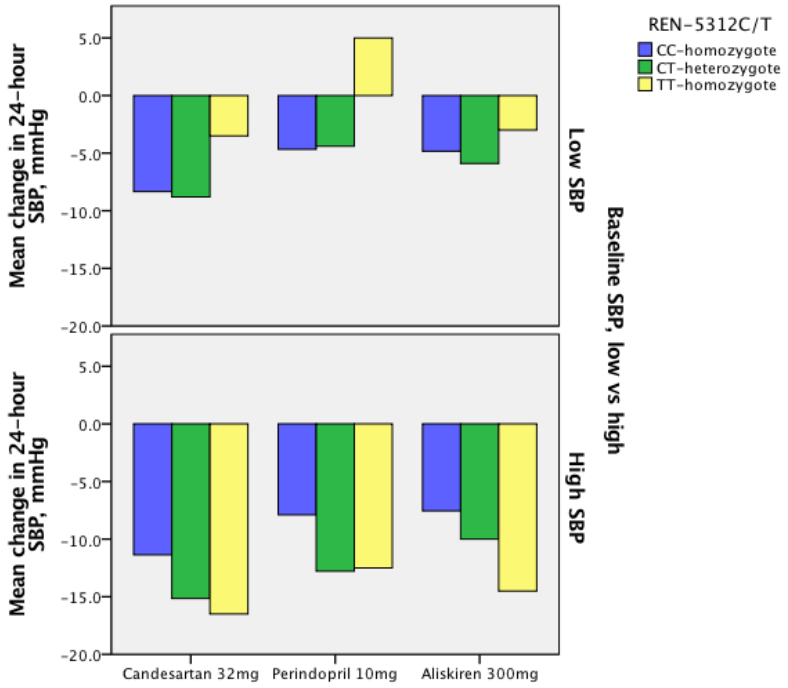
		24 Hour SBP		Daytime SBP		Night-time SBP		24 Hour DBP		Daytime DBP		Night-time DBP	
Factor	Level	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value	Coeff (Std Err)	p-value
Intercept		121.2(20.0)	≤0.0001	130.8(21.7)	≤0.0001	110.9(36.2)	≤0.0001	65.6(11.4)	≤0.0001	66.1(16.1)	≤0.0001	27.5(5.60)	≤0.0001
Age			N/S		N/S		N/S	-0.12(0.05)	0.0206		N/S		N/S
Gender (female)		-6.11(1.43)	≤0.0001	-5.74(1.55)	0.0002	-6.65(1.70)	0.0001	-3.53(0.90)	0.0001	-2.80(0.97)	0.0046	-4.38(1.16)	≤0.0001
Treatment			0.0006		0.0041		0.0450		0.0001		0.0022		0.0482
	Aliskiren	2.08(0.93)	0.0268	2.47(1.00)	0.0145	1.03(1.12)	0.3578	1.37(0.58)	0.0194	1.67(0.65)	0.0109	0.77(0.77)	0.3218
	Candesartan	-3.65(0.93)	0.0001	-3.19(1.00)	0.0018	-2.79(1.13)	0.0137	-2.49(0.58)	≤0.0001	-2.20(0.65)	0.0008	-1.91(0.77)	0.0144
	Perindopril	1.57(0.94)	0.0943	0.72(1.00)	0.4934	1.76(1.13)	0.1216	1.12(0.59)	0.0569	0.53(0.65)	0.4132	1.14(0.78)	0.1442
Baseline BP		-0.96(0.14)	≤0.0001	-1.00(0.15)	≤0.0001	-0.94(0.27)	0.0007	-0.79(0.12)	≤0.0001	-0.82(0.18)	≤0.0001	-0.45(0.07)	≤0.0001
Ln PRA		-7.8(1.96)	0.0004	-8.11(2.10)	0.0005	-5.68(2.34)	0.0160		N/S		N/S		N/S
REN-5312C/T Genotype			0.0048		0.0073		0.0198		0.0151		0.0341		N/S
	TT	116.2(37.2)	0.0018	128.7(40.6)	0.0017	121.7(71.4)	0.0894	57.2(19.7)	0.0040	75.9(31.6)	0.0169		
	CT	-47.4(23.3)	0.0432	-69.6(25.3)	0.0064	-37.4(37.8)	0.3234	-27.5(11.7)	0.0200	-44.2(16.9)	0.0095		
	CC	-68.8(21.8)	0.0018	-59.1(23.3)	0.0118	-84.3(36.8)	0.0228	-29.7(11.3)	0.0090	-31.7(16.8)	0.0602		
REN-5312C/T*Baseline BP Interaction			0.0250		0.0069		0.0166		0.0331		0.0394		N/S
	TT	-0.84(0.27)	0.0019	-0.90(0.28)	0.0016	-0.92(0.54)	0.0904	-0.65(0.22)	0.0051	-0.82(0.35)	0.0196		
	CT	0.34(0.17)	0.0400	0.48(0.17)	0.0056	0.28(0.28)	0.3346	0.31(0.14)	0.0244	0.48(0.19)	0.0112		
	CC	0.50(0.16)	0.0015	0.42(0.16)	0.0098	0.65(0.28)	0.0219	0.34(0.13)	0.0100	0.34(0.19)	0.0646		
REN-5312C/T*Ln PRA Interaction			N/S		N/S		N/S		≤0.0001		≤0.0001		0.0270
	TT							13.57(2.53)	≤0.0001	13.58(2.78)	≤0.0001	8.17(3.08)	0.0084
	CT							-7.01(2.18)	0.0034	-6.39(2.41)	0.0084	-4.89(2.71)	0.0720
	CC							-7.08(1.51)	≤0.0001	-7.19(1.62)	≤0.0001	-3.28(1.87)	0.0797
r squared value		0.28		0.28		0.21		0.34		0.32		0.20	

Figure 4 – Figure illustrating the relationship between antihypertensive effect of each drug and renin -5312C/T genotype.



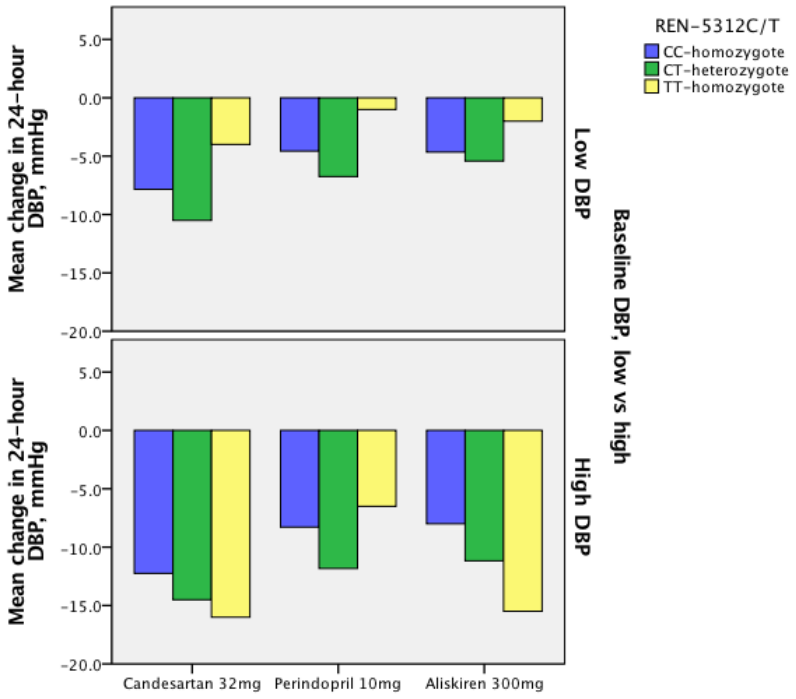
mmHg, millimetres of Mercury; SBP, systolic blood pressure; DBP, diastolic blood pressure

Figure 5 - Figure illustrating mean change in 24-hour systolic blood pressure with each treatment by low or high baseline blood pressure and genotype. TT-homozygotes with high baseline SBP levels achieved greater blood pressure lowering than CC-homozygotes or CT-heterozygotes.



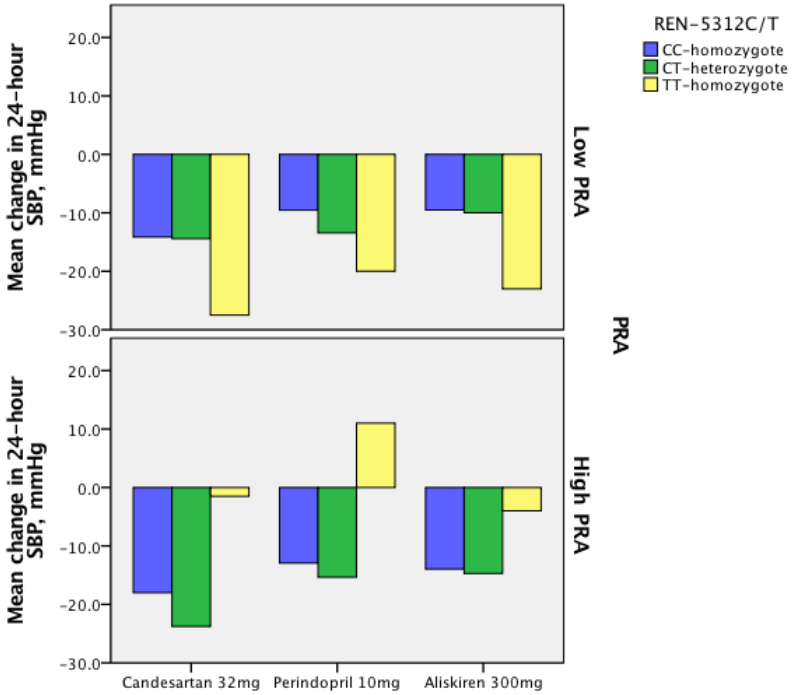
mmHg, millimetres of Mercury; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Figure 6 - Figure illustrating mean change in 24-hour diastolic blood pressure with each treatment by low or high baseline blood pressure and renin genotype.



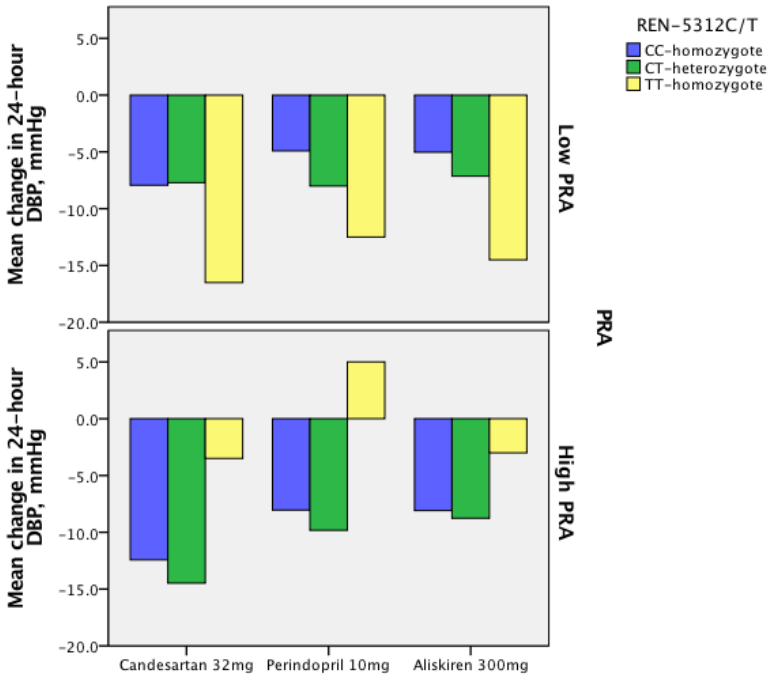
mmHg, millimetres of Mercury; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Figure 7 – Mean change in 24-hour systolic blood pressure with each blocker of the renin angiotensin system by low or high PRA. Low PRA participants achieved greater blood pressure lowering with carriage of REN-5312T.



PRA, plasma renin activity, CC, CC-homozygotes, renin -5312 C/T; CT, CT-heterozygotes, renin -5312 C/T; TT, TT-homozygotes, renin -5312 C/T; PRA, plasma renin activity.

Figure 8 - Mean change in 24-hour diastolic blood pressure with each blocker of the renin angiotensin system by low or high PRA. Low PRA participants achieved greater blood pressure lowering with carriage of REN-5312T, and this effect was greatest for TT-homozygotes. High PRA CT-heterozygotes achieved greater blood pressure lowering than CC-homozygotes, with less blood pressure lowering observed in TT-homozygotes.



CC, CC-homozygotes, renin -5312 C/T; CT, CT-heterozygotes, renin -5312 C/T; TT, TT-homozygotes, renin -5312 C/T; PRA, plasma renin activity

11 SAFETY EVALUATION

Adherence to Medication

Compliance, quantified by counting of returned capsules, was >95% across all treatment groups.

Study Withdrawals

There were eight study withdrawals during the study period. Study withdrawals occurred for the most part with the candesartan use as a result of symptoms related to postural hypotension. Aliskiren was discontinued as a result of GI upset with diarrhoea in one patient. Perindopril caused one subject to withdraw as a result of dry mouth and sore throat. One patient discontinued by choice as a result of the inconvenience and discomfort wearing the 24-hour ABPM machine.

Serious Adverse Events

There were two reported Serious Adverse Events, SAEs, during the study period.

The first was deemed unrelated to study participation, and involved recurrence of an epidermoid cyst on the participant's back that became infected, needing oral antibiotics with inpatient incision and drainage performed. The participant was on aliskiren when this occurred, and study treatment was not affected or interrupted with the patient successfully completing study participation.

The second SAE involved a participant who had completed the clinical trial successfully twenty-one days prior to being admitted to Beaumont Hospital with an ST-segment elevation myocardial infarction (STEMI). This had occurred whilst ascending from a deep-sea dive. The study physician had not been informed of his intention to proceed with deep-sea diving either prior to, during or after study completion. The SAE was not related to his post-trial prescribed antihypertensive medications (amlodipine 10mg once daily and candesartan 32mg once daily). Of note, this participant was of high cardiovascular risk being a smoker, obese and taking statin therapy for dyslipidaemia. His mean 24-hour blood pressure after one month of dual therapy with amlodipine 10mg once daily and candesartan 32mg once daily whilst a study participant had measured 128/75 mmHg, indicating adequate control.

Both SAEs were reported to the Beaumont Hospital Ethics Committee and Irish Medicines Board.

12 DISCUSSION AND OVERALL CONCLUSIONS

Discussion

In this study, blood pressure lowering responses were observed to be greater:

- With candesartan treatment compared with aliskiren or perindopril treatments;
- In females compared to males;
- In participants with higher blood pressures;
- In participants with higher PRA levels (supine PRA of ≥ 1ng/mL/hour);
- In REN-5312T allele carriers who also had higher BP levels and/or lower PRA levels (supine PRA of ≤ 1ng/mL/hour).

Additional findings included:

- An association between REN-5312C/T genotype and PRA;
- An association between REN-5312C/T genotype and serum potassium levels;

Regarding the dose of the antihypertensives used in this current study, the question has been asked whether or not additional blood pressure lowering would have been achieved with higher doses. Perindopril erbumine has been shown to significantly reduce systolic and diastolic blood pressure in patients with mild to moderate hypertension (P. A. P. Todd & Fitton 1991). There appears to be a flattening of the dose response curve at 8mg (Chrysant et al. 1993). Work published in 1996 determined no significant additional blood pressure lowering when the dose of perindopril erbumine was increased from 8mg to 16mg (Myers 1996). Similar changes in systolic and diastolic pressures were observed during the 12-week study period. The analysis included 260 eligible participants randomized in a parallel group design to either placebo or perindopril up to a maximum dose of 16mg daily. We used a slightly different perindopril salt preparation, perindopril arginine, licensed for use in Ireland at the 10mg maximum daily dose. The newer salt preparation has been used preferentially as it possesses greater stability in higher humidities. The change in salt has not been shown to affect the efficacy of perindopril as an antihypertensive with similar bioequivalences, and the lipophilic perindopril has favourable tissue penetration (Fox 2007; Fox & Investigators 2003).

As a class, angiotensin receptor blockers (ARBs) are also better tolerated than ACE inhibitors, with cough and rare but potentially life-threatening angio-oedema experienced by individuals taking the latter (Lacourcière & Asmar 1999). ARBs are highly selective for the angiotensin II receptor, AT_1R. Interesting work examining the binding of valsartan, candesartan and losartan suggested differing binding affinities resulting from differences in the degree of their binding interactions with the AT_1R. Candesartan showed high affinity, valsartan moderate affinity and losartan low affinity binding with the receptor through hydrogen bonds (Bhuiyan et al. 2009).

The candesartan affinity was explained by potentially greater number of binding sites available to the drug given its design. In healthy human volunteers the *in vivo* AT_1R blocking effect of candesartan was shown to be approximately twofold greater than that of losartan with longer duration of action (Belz et al. 1997). Such high affinity for the receptor without doubt has an important influence on the potency of candesartan as an antihypertensive. In this work, it is absolutely possible, with such high affinity binding of candesartan to the AT_1R receptor and subsequent slow dissociation, that candesartan is a better antihypertensive drug pharmacologically than perindopril and aliskiren.

Objective evidence for daily doses greater than 32mg daily is lacking. Supra-maximal doses of candesartan have been previously evaluated in proteinuric renal disease, however not formally assessed in terms of hypertension control. In one 2009 study, 269 diabetic patients with proteinuria were randomized to receive 30 weeks of either candesartan 16mg, 64mg or 128mg once daily. The primary endpoint was reduction in proteinuria and the study achieved statistical significance with candesartan 128mg daily ($P<0.0001$) (Burgess et al. 2009). With blood pressure measurement as a secondary endpoint, the authors stated no statistical significance in blood pressure measured across the three treatment groups. As the effect of the higher dosages of candesartan on blood pressure was a secondary endpoint and not what the study

was primarily designed to evaluate this is difficult to interpret.

A significant dose-dependent effect is seen in terms of the effect of aliskiren on systolic and diastolic blood pressure up to the 300mg dose of aliskiren (P<0.001) (Gradman et al. 2005). However, studies have shown that there is a negligible increase in antihypertensive effect of aliskiren when doses exceed 300mg and are doubled to 600mg (Gradman et al. 2005). In comparison studies, aliskiren 150mg has been compared to the ARBs irbesartan 150mg and losartan 100mg (Gradman et al. 2005; Stanton et al. 2003). The former study determined the antihypertensive effect of aliskiren 150mg to be comparable to that of irbesartan 150mg and indeed significantly superior when aliskiren was increased to 300mg, the dose of irbesartan remaining at 150mg (p<0.05). The latter study demonstrated a comparable blood pressure lowering effect when aliskiren 75mg, 150mg and 300mg were assessed against losartan 100mg (Stanton et al. 2003). An additional factor in terms of dose uptitration to a 600mg dose is that side-effects, in particular gastro-intestinal, are seen to increase when the dose increases from 300mg to 600mg (Weir et al. 2007). Such a significant side-effect would have the potential to impact on quality of life with a subsequent effect on patient compliance.

Gender differences are well acknowledged in both hypertension and cardiovascular disease in general. The way in which the arterial tree ages has been shown to be different between the sexes (Smulyan et al. 2001). Gender differences in various components of the renin-angiotensin system are known to contribute to control of blood pressure, and it is recognised that plasma renin activity is higher in men than women (Kobori et al. 2007; Fischer & Baessler 2002).

In terms of the effect of gender on response to antihypertensive therapy, a 2001 meta-analysis analysed the role of gender differences when controlled-onset extended release verapamil was administered in three prospective, randomized double-blind, placebo-controlled trials. The authors found a greater reduction in 24-hour systolic and diastolic blood pressure for women compared with men (p<0.001 24-hour systolic BP and p=0.003 24-hour diastolic BP) (W. B. White et al. 2001). A further study evaluated the effect of race and gender in a mixed population of participants between 30 and 59 years of age on response to treatment with hydrochlorthiazide. 225 African Americans and 280 Caucasians were randomized to receive hydrochlorthiazide 25mg once daily. Black race and female gender were both associated with significantly greater systolic and diastolic blood pressure lowering with the diuretic treatment. Women of both races achieved greater systolic and diastolic blood pressure lowering than men, P ≤0.01 for both systolic and blood pressures (Chapman et al. 2002). Further still, the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) Study showed that greater systolic and diastolic blood pressure response was seen in females treated with atenolol as monotherapy and as an add-on when compared with response to hydrochlorthiazide (-4.21/0.79 mmHg and -3.73/-2.39 mmHg respectively).

This current study identified the importance of gender in prediction of blood pressure lowering response, with female achieving greater blood pressure than males. It is plausible that significant differences in weight between female and male participants may have contributed to the gender difference observed in this study (Figures 3.14, 3.16). An implication of this may be that drug doses need to be aligned with increase in weight, as higher drug doses may be required for individuals of heavier weight.

Higher baseline blood pressure resulted in greater blood pressure lowering achieved. Baseline blood pressure is a known predictor of response to antihypertensive treatment (Campo et al. 2002). In a study of 225 subjects, a significant correlation was observed between the initial blood pressure and change in blood pressure achieved (Sumner et al. 1988). Patients received either placebo or one of an ACE inhibitor, calcium antagonist, direct vasodilator, α-adrenoceptor blocker or β–adrenoceptor blocker. The change in blood pressure with all treatments including placebo was significantly correlated with initial blood pressure.

Pre-treatment PRA has been advocated as a guide to treatment in hypertensive patients since the 1970s. Patients with higher baseline PRA should theoretically have a correspondingly greater blood pressure reduction when treated with a RAS blocker. This is reflected in how we

manage blood pressure according to age, knowing that younger individuals have more active renin angiotensin systems than older individuals, advancing age associated with a decline in PRA. As such, our first-line drugs in the management of hypertension in individuals in particular under the age of 55 years are RAS blockers. Drugs anticipated as having less of a blood pressure lowering effect in terms of RAS blockade such as amlodipine and diuretics are held in reserve as add-on treatments in those under the age of 55 years. Conversely, hypertensive patients with low pre-treatment PRA will benefit mainly from amlodipine and diuretics, versus specific RAS blockers.

A previous study performed in over 1000 hypertensive individuals assessed baseline PRA and then response to one of six antihypertensive drug classes or placebo. A borderline contribution was seen to the prediction of blood pressure response by PRA, P=0.05 (Preston et al. 1998). Later work performed by Canzanello and colleagues evaluated the validity of measuring pre-treatment PRA in a population of African-American and non-Hispanic white subjects (Canzanello et al. 2008). A higher PRA, >0.65 ng/mL/hr, at the time of enrolment contributed to predicting a greater antihypertensive response to candesartan 32mg daily. After adjusting for other variables, higher PRA was associated with a doubling of the odds of achieving optimum systolic and diastolic BP control with candesartan 32mg. This was most notable in those individuals with higher baseline blood pressure.

In this current study, a threshold of 1ng/mL/hr was assigned to PRA. Above this level patients were considered to have a status of high PRA, and below considered low PRA. Log PRA was consistently statistically significant across all measures of blood pressure as an independent predictor of blood pressure lowering response. We confirmed that high baseline PRA was associated with greater blood pressure lowering.

An interaction was identified between REN-5312C/T genotype and PRA in those with higher baseline blood pressure. These results suggest that this interaction effect may be relevant in guiding antihypertensive therapy. This interaction will potentially allow for stratification of patients into those who will obtain greater blood pressure lowering with RAS blockade. The greatest advantage of genotyping for REN-5312C/T in the future will likely be for moderate to severe hypertensive individuals with low PRA. We would expect that CT-heterozygotes and TT-homozygotes with moderate to severe hypertension and low PRA will achieve greater blood pressure lowering with RAS blockade, particularly with potent angiotensin receptor blockers such as candesartan. The mechanism for the interaction effect between PRA and the renin genotype is unclear, but perhaps related to varying levels of tissue or circulating RAS components.

We acknowledge the limited predictive effect of one SNP on blood pressure. The interaction observed here could therefore in future constitute one of a wider panel of SNPs tested to determine the most appropriate antihypertensive therapy for an individual. This is of relevance as we continue to move forward in an era of personalised medicine, with less trial and error approach applied and a focus on the “right treatment, for the right person, at the right time” (Scholz 2015).

In a previous study performed by our group, 257 individuals were recruited to a randomised, double-blind, active-comparator, parallel group blood pressure lowering trial comparing response to aliskiren or losartan by REN-5312C/T genotype (Moore et al. 2007). In their study, T-allele carriers on losartan achieved greater blood pressure lowering than CC-homozygotes. We have confirmed their results in this current work. In addition, in this present study, REN-5312C/T genotype was seen to influence supine PRA (p=0.03). The mechanism by which the SNP did this is unclear, but supine rather than upright PRA is acknowledged to be a more accurate measure of circulating renin when sampling (Lonati et al. 2014).

Significantly higher baseline potassium levels were observed in TT-homozygotes (mean 4.4 mmol/L) when compared with CC-homozygotes and CT-heterozygotes (mean 4.0 mmol/L) (p=0.02). Importantly however, the results observed were still within normal range (3.5 – 5 mmol/L), and so the clinical significance of this is not clear, warranting further evaluation. There was no convincing evidence of renal impairment in TT-homozygotes, and the mechanism for

this is unclear. Possible theories include an effect of REN-5312C/T on kidney sodium and potassium co-transporters in the thick ascending limb of the loop of Henle (Na^+-K^+-$2CL^-$), or an effect on serum aldosterone levels. No significant differences in serum sodium or chloride were observed amongst the genotypes however. It could be considered to measure aldosterone alongside renin in a future study to explore this further.

Conclusions

The data from this pharmacogenetic clinical trial observed that blood pressure lowering responses were greater:

- With candesartan treatment compared with aliskiren or perindopril treatments;
- In females compared to males;
- In participants with higher blood pressures;
- In participants with higher PRA levels;
- In REN-5312T allele carriers who also had higher BP levels and/or lower PRA levels.

REN-5312C/T may yet become a genotype used in practice to identify patients who will gain maximal benefit from RAS blockade both in terms of blood pressure lowering and protection from end-organ damage. It is doubtful that the REN-5312C/T genotype alone will act as a useful predictor of antihypertensive response. It is much more likely that REN-5312C/T will direct treatment as one of a panel of genes identified as having an impact on prediction of response to antihypertensive therapy.

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14 APPENDICES

9.1 Study Protocol

9.2 Participant Information Leaflet

9.3 Participant Consent Forms

9.4 Ethics Committee Approval

9.5 Regulatory Approval

Appendix 14.1

Study Protocol

Protocol Title:
Renin Genotype and Response to Renin Angiotensin System Blockade.

Protocol Code No:
RGR 001

Protocol version:
Version 3 29/07/2008

EudraCT number:
2008-003568-20

Principal Investigator:
(Single centre trial)
Prof. Alice Stanton, Molecular and Cellular Therapeutics, RCSI Research Institute, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2, Ireland.

Sponsor:
Prof. Alice Stanton, Molecular and Cellular Therapeutics, RCSI Research Institute, Royal College of Surgeons in Ireland, St. Stephen's Green, Dublin 2, Ireland.

Other relevant personnel:
Co-Investigators
Dr. Brendan McAdam & Dr. Ursula Quinn, Department of Cardiology, Beaumont Hospital, Dublin 9, Ireland.

Study site(s);
Department of Cardiology, Blood Pressure Unit, and RCSI Clinical Research Centre, Beaumont Hospital, Dublin 9, Ireland.

Protocol Approval Signature Page

I, the principal investigator of this study, will ensure that this study is conducted in accordance with the study protocol here provided, (subject to any amendments) in compliance with Good Clinical Practice, the EU CT Directive 2001/20/EC, and with all other local and international applicable regulatory requirements.

Prof. Alice Stanton	Signed	
Principal Investigator:	Date	29th July 2008

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3 LIST OF ABBREVIATIONS

ABPM	Ambulatory Blood Pressure Monitoring
ACE	Angiotensin converting enzyme
AE	Adverse Event
ADR	Adverse Drug Reaction
BP	Blood Pressure
CRF	Case Report Form
CBPM	Clinic Blood Pressure Measurement
DSMB	Data and Safety Monitoring Board
ECG	ElectroCardioGram
GCP	Good Clinical Practice
GP	General Practitioner
MORGAM	MOnica Risk Genetics Archiving and Monograph project
PRA	Plasma Renin Activity
RAS	Renin Angiotensin System
REN	Renin gene
SADR	Serious Adverse Drug Reaction
SAE	Serious Adverse Event
SD	Standard Deviation
SE	Standard Error
SNP	Single Nucleotide Polymorphism
SUSAR	Suspected Unexpected Serious Adverse Reaction
UADR	Unexpected Adverse Drug Reaction

4 STUDY SYNOPSIS

Title	Renin Genotype and Response to Renin Angiotensin System Blockade.
Investigational Medicinal Product(s)	Aliskiren 300 mg daily, Candesartan 32 mg daily, Perindopril 10 mg daily. Amlodipine 10 mg (concomitant medication)
Study Objectives:	To confirm that genotyping of the REN-5312C/T polymorphism identifies individuals who have different BP lowering responses to particular blockers of the renin angiotensin system, and therefore validate REN-5312C/T genotyping as a useful pharmacogenetic assay. Specific primary objectives are; 1. To compare the BP lowering responses to a renin inhibitor, an angiotensin receptor blocker, and an angiotensin converting enzyme inhibitor, amongst REN-5312 CC homozygotes. 2. To compare the BP lowering responses to a renin inhibitor, an angiotensin receptor blocker, and an angiotensin converting enzyme inhibitor, amongst REN-5312 T allele carriers. A secondary objective is to store DNA and clinical data so as to allow further appropriate pharmacogenetic analyses.
Study Design:	A phase IV single centre prospective randomized open cross-over pharmacogenetic clinical trial comparing BP lowering responses to a renin inhibitor (aliskiren), an angiotensin receptor blocker (candesartan), and an angiotensin converting enzyme inhibitor (perindopril), amongst REN-5312 T allele carriers and amongst REN-5312 CC homozygotes.
Inclusion Exclusion Criteria	100 patients with essential hypertension will be recruited to this trial. *Inclusion Criteria:* • Male or female outpatients • Age greater than 18 years. • Written informed consent provided. • Patients with essential hypertension who are either antihypertensive treatment naïve, or taking a maximum of two antihypertensive agents. • Baseline mean 24-hour systolic pressure > 130 mm Hg, and < 160 mm Hg, either on no treatment or on amlodipine 10 mg daily. *Exclusion criteria:* • Age less than 18 years old. • Pregnancy, women who are breast feeding, or with childbearing potential without using a medically accepted method of contraception.

	• Presence of any significant acute or chronic illness. In particular participants must not have a history of an acute cardiovascular morbid event within the last 3 months. They must not have suffered with malignant hypertension or congestive heart failure. They must not have a terminal illness.
	• Significant baseline electrolyte, serum creatinine or creatinine clearance abnormalities (potassium > 5.5 mmol/l, or serum creatinine > 150 micromol/l, or creatinine clearance < 30mls/minute as assessed by Cockcroft's formula)
	  ○ Men = (140 - age) X weight (kg) / (0.814 X creatinine level (micromol/l)
	  ○ Women = 0.85 X [(140 - age) X weight (kg) / (0.814 X creatinine level (micromol/l)]
	• Known secondary hypertension
	• Requirement for any specific antihypertensive drug therapy.
	• Patients already taking three or more antihypertensive agents - alpha-blockers, when prescribed for indications other than hypertension, are not counted as an antihypertensive agent in this regard.
	• Patients unable to discontinue current antihypertensive therapy - alpha-blockers already prescribed for indications other than hypertension, at a dose which will not change during the study, may be continued.
	• Contra-indications to any of the study drugs.
	• Participation in any other studies involving investigational or marketed products within one month prior to entry into this study or concomitantly with this study.
	• Participants that are unlikely to comply well with study treatments or with the scheduled visits.
	• Participants with a history of alcohol or drug abuse, psychosis, antagonistic personality, or any emotional or intellectual problems that are likely to invalidate informed consent, or limit the ability of the subject to comply with the protocol requirements.

Primary and Secondary Efficacy Endpoints:	The primary efficacy end-point will be the change from baseline in 24-hour systolic BP with each drug treatment. Secondary efficacy end-points; • change from baseline in 24-hour diastolic BP • change from baseline in daytime systolic BP • change from baseline in daytime diastolic BP • change from baseline in night-time systolic BP • change from baseline in night-time diastolic BP • Proportion of participants with controlled BP, defined as mean 24-hour systolic BP < 130mmHg and mean 24-hour diastolic BP < 80mmHg • change from baseline in clinic systolic BP • change from baseline in clinic diastolic BP
Safety Endpoints:	• Adverse events • Adverse drug reactions • Worsening of hypertension • Symptomatic hypotension • Electrolyte abnormalities • Deterioration in renal function (serum creatinine and creatinine clearance)

5 BACKGROUND INFORMATION

Heart attacks and strokes account for approximately 40% of deaths world wide, and are the leading cause of disease burden. Almost half of heart attacks and strokes are due to uncontrolled hypertension (elevated blood pressure). Currently control of hypertension is very poor – less than 25% of patients with hypertension have their BP at or below target levels. The considerable variation in inter-individual response to currently prescribed antihypertensive drugs certainly contributes to this exceedingly poor control rate - up to 50% of patients gain little or no BP lowering with any particular drug class.

The renin angiotensin system (RAS) plays important roles in the regulation of electrolytes, blood pressure (BP) and atherosclerosis.[1,2] Renin catalyses the first and rate limiting step of this cascade, the conversion of angiotensinogen to angiotensin I. Hypertensive patients with high plasma renin levels are more likely than those with normal or low renin levels to experience myocardial infarctions.[3] Furthermore BP lowering responses to antihypertensive drugs differ depending on the plasma renin status of the patient.[4] Current international guidelines (US, European and British) all include a blocker of the RAS as first-line or second-line therapy for hypertension. Furthermore inhibition of the RAS is indicated for a wide range of patients with cardiovascular disease - those who have suffered a stroke or myocardial infarction, and also those with diabetes mellitus and nephropathy.

A common single nucleotide polymorphism (SNP) in a renin distal enhancer element (REN-5312C/T) has been reported to influence *in vitro* gene transcription in transfected human choriodecidual cells[5] – in 2002 Fuchs and colleagues noted 45% greater rates of renin gene transcription in the presence of a -5312T allele rather than a -5312C allele.[6]

Even more recently, Stanton and colleagues provided the first evidence that this REN–5312C/T polymorphism has *in vivo* functional activity in humans.[7] Carriage of the -5312T allele, a specific marker for a single renin haplotype, was found to be associated with both elevated ambulatory and elevated clinic blood pressure (BP) levels in healthy Allied Irish Bank employees. The magnitude of the effect associated with carriage of the -5312T allele ranged from 2.7 mm Hg to 1.5 mm Hg.[7]

Furthermore, her group also found evidence that the polymorphism predicts BP lowering responses to RAS blockade in hypertensive patients, and that this prediction is additional to, and independent of plasma renin activity (PRA). Blood pressure lowering with an angiotensin receptor blocker (losartan 100 mg daily), among T allele carriers with a baseline PRA greater than the median value, was more than twice that of CC homozygotes with a baseline PRA less than the median. While BP responses with a renin inhibitor (aliskiren 150 mg or 300 mg daily), were also positively correlated with baseline PRA, BP lowering, particularly at night, was considerably greater amongst CC homozygotes compared to T allele carriers, (-10.1(1.4)/-6.5(1.1) versus -5.4(2.0)/-4.1(1.3), $p<0.03$ for treatment*genotype interaction for night-time systolic and diastolic pressures).[7] These findings, of independent and disparate predictions of responses to renin inhibition and to angiotensin receptor blockade, by baseline PRA and by

renin -5312C/T genotype, suggest that there may be clinical utility in measuring both PRA and renin genotype, prior to the prescription of antihypertensive therapy. Furthermore the divergent BP lowering responses with the two blockers of the RAS may be paralleled by cardiovascular protection.

Interestingly, in the same group of hypertensive patients, no difference was found in PRA at baseline between renin -5312T allele carriers and CC homozygotes. Plasma renin activity of T allele carriers was neither more resistant to suppression by aliskiren nor more elevated with losartan therapy than that of CC-homozygotes. The associations of genotype and baseline PRA, with BP lowering responses to renin inhibition and to angiotensin receptor blockade, were independent of each other.[7] These three findings suggest that the renin -5312C/T polymorphism does not influence the highly regulated secretion of active renin from renal juxtaglomerular cells into the systemic circulation. It is noteworthy that mice with two renin genes do demonstrate higher BP levels, but have lower levels of both plasma and renal renin, than mice with only one renin gene.[8] Hence a plausible explanation is that functionality of the renin -5312C/T polymorphism is mediated by altered local tissue or even intracellular renin or prorenin levels.[9,10] In keeping with this suggestion was an observation of a non-significant trend for aliskiren levels to be lower in T allele carriers. This led to the speculation that high affinity binding of aliskiren by elevated tissue renin in -5312T allele carriers could have contributed to the lesser BP lowering achieved in these individuals after 4 weeks of aliskiren. Alternatively or additionally, a failure by aliskiren to inhibit the catalytic activity or the intracellular signalling that occurs when renin or prorenin binds to the recently discovered (pro)renin receptor, could have contributed to the lesser BP lowering seen in renin -5312T allele carriers with aliskiren.[11,12]

Stanton and colleagues have gone on to confirm the association of renin -5312C/T genotype with BP level in a second larger population (unpublished data). Her group are currently testing whether the polymorphism predicts future cardiovascular morbidity and mortality in a large European cohort collaborative study, the MOnica Risk Genetics Archiving and Monograph (MORGAM) project.[13]

Hence the principal hypothesis that will be tested in this pharmacogenetic clinical trial is that renin -5312C/T genotype predicts BP lowering responses to RAS blockade. To that end, BP lowering responses to three blockers of the renin angiotensin system, according to renin genotype, will be studied amongst patients with mild to moderate essential hypertension. The three blockers of the renin angiotensin system, that will be studied will be the only renin inhibitor currently licensed for use in Europe, aliskiren, an angiotensin receptor blocker, candesartan, and an angiotensin converting enzyme inhibitor, perindopril. All three drugs will be administered orally, once a day. Participants will receive each drug for four weeks. Dosages of all three will be the maximum maintenance dosages recommended for the treatment of essential hypertension.

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6 OBJECTIVES OF THE TRIAL

Primary:	To confirm that genotyping of the REN-5312C/T polymorphism identifies individuals who have different BP lowering responses to particular blockers of the renin angiotensin system, and therefore validate REN-5312C/T genotyping as a useful pharmacogenetic assay. Hence the specific primary objectives of this trial are; 1. To compare the BP lowering responses to a renin inhibitor, an angiotensin receptor blocker, and an angiotensin converting enzyme inhibitor, amongst REN-5312 CC homozygotes. 2. To compare the BP lowering responses to a renin inhibitor, an angiotensin receptor blocker, and an angiotensin converting enzyme inhibitor, amongst REN-5312 T allele carriers.
Secondary:	To store DNA and clinical data so as to allow further appropriate pharmacogenetic analyses.

7 STUDY DESIGN

This will be a phase IV single centre prospective randomized open cross-over pharmacogenetic clinical trial comparing BP lowering responses to a renin inhibitor (aliskiren), an angiotensin receptor blocker (candesartan), and an angiotensin converting enzyme inhibitor (perindopril), amongst REN-5312 CC homozygotes, and amongst REN-5312 T allele carriers.

Study Plan

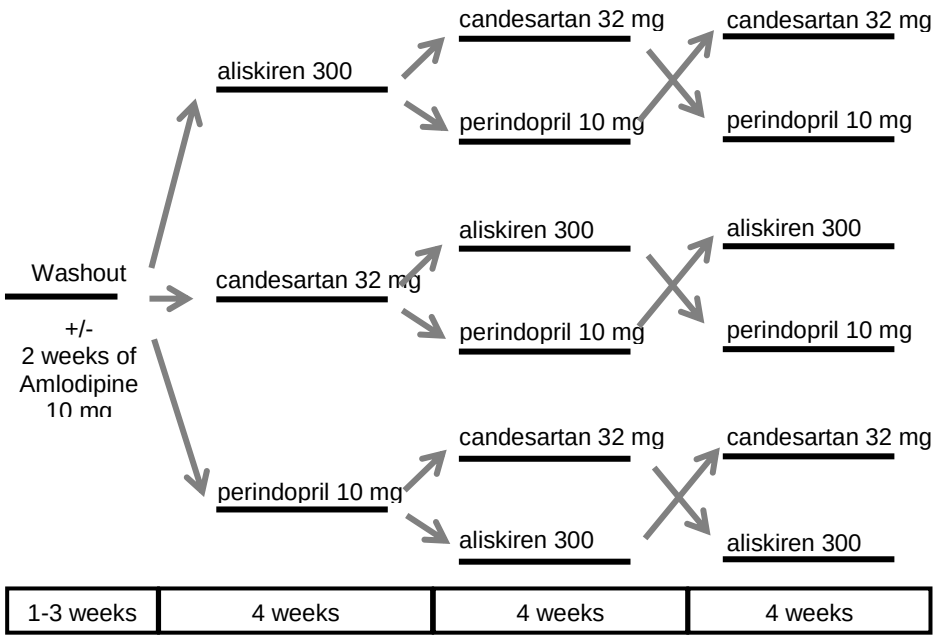
Written informed consent will be obtained from all participants prior to entry into the study. In obtaining informed consent the study investigator will provide the potential participant with information about the purposes, methods, possible risks and benefits of participating in the study. All potential participants will have an opportunity to discuss the study with study staff. The participant and the person obtaining informed consent will each sign and date two copies of the consent form, one copy of which will be provided to the participant, and the other copy of which will be stored in the participant's case record folder. Participation in the study will be voluntary, and all participants will be free to withdraw at any time. If the participant is agreeable, a letter, describing the study and their participation, will be sent by the study physician to the participant's General Practitioner (GP).

At the screening visit a comprehensive medical history and physical examination will be performed.

After one week free of all vasoactive medications, clinic BP and 24-hour ambulatory BP will be measured. Erect and supine phlebotomy will be performed - this will allow the following; measurement of serum urea, electrolytes and creatinine estimation (from supine sample only); measurement of plasma renin activity; Immediate spinning down of blood samples and storage of plasma aliquots for future measurements (currently undetermined); DNA extraction for REN-5312C/T genotyping, and storage of DNA for further genetic studies (from supine sample only).

Patients whose baseline mean 24-hour systolic pressure is $\geq$ 130 mm Hg, and $\leq$ 160 mm Hg are eligible for inclusion. Those patients whose baseline mean 24-hour systolic pressure is > 160 mm Hg and $\leq$ 175 mm Hg, will be prescribed amlodipine 10 mg daily, and 24-hour ambulatory BP will be repeated 2 weeks later. If mean 24-hour systolic pressure is now $\geq$ 130 mm Hg, and $\leq$ 160 mm Hg, these patients are eligible for inclusion, and amlodipine 10 mg will be continued throughout the clinical trial.

All participants will then be allocated, in random order (stratified according to amlodipine usage or not), to 4 weeks of treatment with a renin inhibitor (aliskiren 300 mg daily), an angiotensin receptor blocker (candesartan 32 mg daily) and an angiotensin converting enzyme inhibitor (perindopril 10 mg daily) See below schema.



Randomisation will be fixed, balanced and organised through use of sealed envelopes.

At the end of each 4 week treatment period clinic BP, 24-hour ambulatory BP and serum urea, electrolytes and creatinine will be measured. Adverse events and concomitant disease activity will be evaluated. Details of all concomitant treatments will be recorded. Patients will be instructed to notify the investigator about any new medications taken after the start of the clinical trial.

At study end, patients will be prescribed the medication(s) which resulted in the best BP control during the study, without resulting in any clinically relevant side effects or adverse events. If the participant is agreeable, a letter, summarising the patient's participation in the study, will be sent by the study physician to the participant's GP

Start date	July 2008 Study duration for each participant = 4 months

End of study date	June 2010

8 SPECIFIC STUDY PROCEDURES

Medical history and Examination

Medical history and examination will include the following assessments; Current diagnoses; Past medical history; Current medications; Lifestyle assessment including diet and smoking history; Family history; Height, weight and waist circumference measurements; Cardiovascular examination

Electrocardiography (ECG)

A standard 12 lead ECG will be performed at screening.

Phlebotomy,

Most blood sampling will occur in the sitting position and 10 mls will be drawn. However at baseline, erect and supine phlebotomy will be performed - blood samples will be taken after 10 minutes in the erect position and after 20 minutes in the supine position. In total 60 mls will be drawn at the baseline visit, 15 mls in the erect position, and 45 mls in the supine position.

Immediate Blood Assays

Serum urea, electrolytes and creatinine will be quantified at baseline and at the end of each treatment period. At the baseline visit only, PRA will be measured from erect and supine blood samples. PRA will be measured by trapping of generated angiotensin I by antibodies and by subsequent radioimmunoassay [14].

Blood Sample Storage, DNA Extraction and Future Assays

At the baseline visit only, erect and supine blood samples will be immediately spun down, serum will be pipetted off and four 1 ml plasma aliquots will be stored at -70^{O}C so as to allow future measurements of bioactive molecules – the specific assays that will be performed are currently undetermined.

At the baseline visit only, whole blood samples preserved in EDTA from the baseline visit will be stored at -70^{O}C so as to allow later DNA extraction for REN-5312C/T genotyping, and storage of DNA for further genetic studies

Clinic Blood Pressure Measurement:

Sitting clinic BP measurement (CBPM) and heart rate (HR) will be measured from the right arm using a regularly calibrated validated automated sphygmomanometer (Omron HEM-705CP). After at least 5 minutes in the sitting position, three measurements will be made at one minute intervals. CBPM and HR will be taken as the average of the second and third readings.

Ambulatory Blood Pressure Measurement:

Ambulatory Blood Pressure Measurements (ABPM) will be made every half-hour throughout the 24-hour period using SpaceLabs 90207 monitors (SpaceLabs Medical Inc. Issaqua, West Virginia, USA). Monitoring will commence between 07.00 and 14.00 hours. Both study medication and concomitant medication will be taken at the usual timing on the days where ABPM is performed. Mean 24-hour, daytime (0900 and 2100 hours), and night-time (0100 and 0600 hours) systolic and diastolic blood pressures, and also heart rates will be calculated from the ABPMs. ABPM will be regarded as satisfactory if there were at least 14 daytime readings and 8 night-time readings. The first study medication will not be commenced until the baseline ABPM has been judged to be satisfactory. The first, second and third study medications will be continued until each end-of-treatment ABPM has been judged to be satisfactory. This is so as to allow a single repetition of each ABPM where the first ABPM is not satisfactory.

Investigation Schedule

	Week -1 or -3 Screening	Week -2		Week 0 Baseline		Week 4		Week 8		Week 12	
	V1	Extra visits *		V2	V3	V4	V5	V6	V7	V8	V9
Informed consent	X										
Medical history	X										
Physical examination	X										
ECG	X										
Inclusion/exclusion criteria			X		X						
Urea, electrolytes and creatinine estimation				X		X		X		X	
Erect and supine PRA				X							
Erect and supine Serum storage				X							
Blood storage for DNA extraction and storage and REN-5312C/T genotyping				X							
Clinic BP measurement	X	X		X		X		X		X	
Placement of ABPM monitor		X		X		X		X		X	
Removal of ABPM monitor					X		X		X		X
Adverse events			X		X		X		X		X
Assessment of concomitant diseases and medications	X		X		X		X		X		X
Dispensing of study medication			X		X		X		X		
Assessment of compliance with study medication					X		X		X		X

***** Two extra visits will be required for those participants whose off treatment mean 24-hour systolic pressure is > 160 mm Hg and < 175 mm Hg. These participants will be prescribed amlodipine 10 mg daily, and a second baseline visit scheduled for 2 weeks later.

Visit windows will be at the designated timing plus/minus one week. Hence the minimum and maximum duration of treatment on each RAS blocker will be 21 and 35 days respectively.

9 PARTICIPANTS

The participants will be 100 patients with mild to moderate essential arterial hypertension. All participants will be attending Beaumont Hospital cardiovascular out-patient clinics.

Women of childbearing age are eligible for inclusion, but only if not pregnant nor breast feeding, and such women must be using a medically accepted method of contraception,

Children (under the age of 18 years) are not eligible for inclusion.

There is no upper age limit to this study, as the prevalence of hypertension increases with age. Furthermore, there is no upper age limit to the benefits of BP lowering. However, the close monitoring for worsening hypertension, symptomatic hypotension, electrolyte disturbances and deteriorating renal function (all described in detail later) will be of great importance in ensuring the safety of more vulnerable elderly patient participants

10 INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria:

- Male or female outpatients
- Age greater than 18 years.
- Written informed consent provided.
- Patients with essential hypertension who are either antihypertensive treatment naïve, or taking a maximum of two antihypertensive agents. .
- Baseline mean 24-hour systolic pressure > 130 mm Hg, and < 160 mm Hg, either on no treatment or on amlodipine 10 mg daily.

Exclusion criteria:

- Age less than 18 years old.
- Pregnancy, women who are breast feeding, or with childbearing potential without using a medically accepted method of contraception.
- Presence of any significant acute or chronic illness. In particular participants must not have a history of an acute cardiovascular morbid event within the last 3 months. They must not have suffered with malignant hypertension or congestive heart failure. They must not have a terminal illness.

- Significant baseline electrolyte, serum creatinine or creatinine clearance abnormalities (potassium > 5.5 mmol/l, or serum creatinine > 150 micromol/l, or creatinine clearance < 30mls/minute as assessed by Cockcroft's formula)
    - Men = (140 – age) X weight (kg) / (0.814 X creatinine level (106icromole/l)
    - Women = 0.85 X [(140 – age) X weight (kg) / (0.814 X creatinine level (micromole/l)]
- Known secondary hypertension
- Requirement for any specific antihypertensive drug therapy.
- Patients already taking three or more antihypertensive agents - alpha-blockers, when prescribed for indications other than hypertension, are not counted as an antihypertensive agent in this regard.
- Patients unable to discontinue current antihypertensive therapy - alpha-blockers already prescribed for indications other than hypertension, at a dose which will not change during the study, may be continued.
- Contra-indications to any of the study drugs.
- Participation in any other studies involving investigational or marketed products within one month prior to entry into this study or concomitantly with this study.
- Participants that are unlikely to comply well with study treatments or with the scheduled visits.
- Participants with a history of alcohol or drug abuse, psychosis, antagonistic personality, or any emotional or intellectual problems that are likely to invalidate informed consent, or limit the ability of the subject to comply with the protocol requirements

11 INVESTIGATIONAL MEDICINAL PRODUCTS (IMP)

All study drugs will be supplied as commercially available tablets.

Study drugs will stored in a secured area with restricted access, at room temperature.

The dosages of the three RAS blockers and the calcium antagonist are the maximum maintenance dosages recommended for treatment of uncomplicated hypertension.

Study treatments will be dispensed by a study investigator (AS, BMcA or UQ) or his/ her deignee, to the patients in accordance with the study protocol, and in accordance with the individual randomization schedule.

The Investigator or appropriate designee will ensure that:

- details of the dispensing and return of all investigational medicinal product are documented on a study dispensing log
- the study dispensing logs are maintained complete, current and accurate
- unused investigational medicinal product are sent for destruction at the end of the study and a final inventory completed and filed in the Investigator Study File

The IMP supplied is for the use of subjects recruited into this study only. It will be the Investigator's responsibility to ensure that these drugs are not under any circumstances loaned or dispensed to other medical staff or site.

Identity and Labelling of Investigational Product(s)
The investigational product for each patient for each patient will be supplied in a standard commercial medication box, comprising medication sufficient for 4 weeks of treatment and 1 week overage. The medication box will be labelled with a unique identifier, which will correspond to a randomization number. The medication box requires storage below 25oC and the medication has to be protected from light and moisture

Sample Drug Labels

Contents: 35 Aliskiren 300 mg Tablets **Lot:** xxx **Expiry Date:** MMM / YYYY **Sponsor & Principal Investigator**: Prof Alice Stanton **Site:** RCSI Clinical Research Centre, Beaumont Hospital, Dublin 9, Ireland. Tel 01 8092862 **Study No:** RGR001 **Subject No: _____ Randomization No:** **Date dispensed : _____ Week:** **Directions for use:** Please take one tablet per day in fasting condition. Store below 25^{o}C. Protect from light and moisture. **Keep out of reach of children** **For clinical trial use only**	

Contents: 75 Candesartan Tablets **Lot:** xxx **Expiry Date:** MMM / YYYY **Sponsor & Principal Investigator**: Prof Alice Stanton **Site:** RCSI Clinical Research Centre, Beaumont Hospital, Dublin 9, Ireland. Tel 01 8092862 **Study No:** RGR001 **Subject No: _____ Randomization No:** **Date dispensed : _____ Week:** **Directions for use:** Please take two tablets per day in fasting condition. Store below 25^{o}C. Protect from light and moisture. **Keep out of reach of children** **For clinical trial use only**	

Contents: 35 Perindopril Arginine 10 mg Tablets **Lot:** xxx **Expiry Date:** MMM / YYYY **Sponsor & Principal Investigator**: Prof Alice Stanton **Site:** RCSI Clinical Research Centre, Beaumont Hospital, Dublin 9, Ireland. Tel 01 8092862 **Study No:** RGR001 **Subject No: _____ Randomization No:** **Date dispensed : _____ Week:** **Directions for use:** Please take one tablet per day in fasting condition. Store below 25^{o}C. Protect from light and moisture. **Keep out of reach of children** **For clinical trial use only**	

Dosage forms and strengths:	Aliskiren 300 mg (Novartis)
Dose Schedule:	1 tablet daily
Route of Administration:	Oral
Duration of treatment:	The minimum and maximum duration of treatment will be 21 and 35 days respectively.

Dosage forms and strengths:	Candesartan 32 mg (AstraZenica)
Dose Schedule:	2 tablets daily
Route of Administration:	Oral
Duration of treatment:	The minimum and maximum duration of treatment will be 21 and 35 days respectively.

Dosage forms and strengths:	Perindopril Arginine 10 mg (Servier)
Dose Schedule:	1 tablet daily
Route of Administration:	Oral
Duration of treatment:	The minimum and maximum duration of treatment will be 21 and 35 days respectively.

12 CONCOMITANT MEDICATION

Patients whose baseline off antihypertensive treatment mean 24-hour systolic pressure is > 160 mm Hg and ≤ 175 mm Hg, will be prescribed amlodipine 10 mg daily, and 24-hour ambulatory BP will be repeated 2 weeks later. If mean 24-hour systolic pressure is now ≥ 130 mm Hg, and ≤ 160 mm Hg, these patients are eligible for inclusion, and amlodipine 10 mg will be continued throughout the clinical trial.

Dosage forms and strengths:	Amlodipine 10 mg (Pfizer)
Dose Schedule:	1 tablet daily
Route of Administration:	Oral
Duration of treatment:	14 weeks

Contents: 35 Amlodipine 10 mg Tablets **Lot:** xxx

Expiry Date: MMM / YYYY

Sponsor & Principal Investigator: Prof Alice Stanton

Site: RCSI Clinical Research Centre, Beaumont Hospital, Dublin 9, Ireland. Tel 01 8092862

Study No: RGR001

Subject No: _____ **Randomization No:**

Date dispensed : _____ **Week:**

Directions for use: Please take one tablet per day in fasting condition. Store below 25°C. Protect from light and moisture.

Keep out of reach of children

For clinical trial use only

13 TREATMENT COMPLIANCE

Throughout the trial, patients will be asked to take all study medication, once daily, 30 minutes prior to eating breakfast. The numbers of capsules/tablets dispensed at each visit, and the numbers returned at each visit will be counted and recorded. This will allow compliance to be automatically calculated. If compliance is unsatisfactory (less than 80%), the participant will be questioned to identify the reason for this.

14 SAFETY

Only participants that satisfy the inclusion and exclusion criteria will be eligible to participate in this study. Hence only volunteers that have hypertension, and are free of any other significant cardiovascular or systemic disease will be exposed to study treatments. All study drug treatments are licensed for use in the management of hypertension.

The following safety measurements will be checked and recorded at each visit.

- Adverse events
- Assessment of concomitant diseases
- Clinic BP and HR
- Ambulatory BP monitoring
- Urea, electrolytes and creatinine estimation

Withdrawal Criteria

Study treatments will be permanently discontinued for a participant for any of the following reasons;

- Onset of an adverse event which presents a risk to the patient, or which requires a prescription of a treatment which is prohibited by the protocol
- Renal function impairment with a decrease in creatinine clearance by >30% as assessed by Cockcroft's formula
    - Men = (140 - age) X weight (kg) / (0.814 X creatinine level (micromol/l)
    - Women = 0.85 X [(140 - age) X weight (kg) / (0.814 X creatinine level (micromol/l)]
- Increased potassium level > 5.5 mmol/l, confirmed in a repeated sample assayed within 7 days.
- Pregnancy
- Worsening of hypertension, defined as mean 24-hour ambulatory systolic BP > 160 mmHg.
- Non-medical reason (patient's personal decision to stop treatment).

15 DEFINITION OF ADVERSE AND SERIOUS ADVERSE EVENTS

An **Adverse Event (AE)** is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.A **Serious Adverse Event (SAE)** is defined as any untoward medical occurrence that;

- results in death,
- is life-threatening, (The patient is at a risk of death at the time of the event. It doesn't refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalisation or prolongation of existing hospitalisation, (Hospital admissions and/or surgical operations planned before or during a study are not considered adverse events if the illness or disease existed before the patient was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study)
- results in persistent or significant disability/incapacity,
- is a congenital anomaly/birth defect, or
- is an important medical event. (Other important events may jeopardise the patient or subject and may require medical or surgical intervention to prevent one of the other serious outcome listed in the definition. (example: blood dyscrasias (eg. neutropenia or anemia requiring blood transfusion etc.) or convulsions that do not result in hospitalisation.)

16 PROCEDURES FOR MONITORING AND RECORDING ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

All adverse events occurring during the study period, regardless of severity, will be recorded. The investigator will complete the following details; dates of onset, severity, corrective therapies given, action taken, outcome and opinion as to whether the AE is likely to be drug-related. Each event will be recorded separately in the Case Report Form.

Any AEs observed during the study (i.e. V1 through to V9) will be followed up to resolution. Resolution means that the patient has returned to a baseline state of health or the investigator does not expect any further improvement or worsening of the adverse event.

The Investigator will report all SAE's to Beaumont Hospital Ethics Committee and also to the Irish Medicines Board within the required period. The investigator will also immediately report any overdose of study drugs and any pregnancies occurring during the study.

17 STATISTICAL ANALYSIS PLAN

The primary end-point will be the change from baseline in mean 24-hour systolic BP with each drug treatment.

Statistical Analyses

Repeated measures ANOVA will be used to compare BP lowering responses to a renin inhibitor, an angiotensin receptor blocker, and an angiotensin converting enzyme inhibitor, amongst REN-5312 CC homozygotes, and amongst REN-5312 T allele carriers. The analysis will be adjusted for age, sex, baseline PRA, and amlodipine usage.

Sample size and study power

Assuming a standard deviation of 6 mmHg for the change in baseline to end-of-treatment 24-hour systolic BP, a significance level of 0.05, and 90 evaluable patients (30 REN-5312 T allele carriers and 60 CC homozygotes), this study will have 80% power to detect 4 mm Hg differences in the primary end-point (change from baseline to end-of-treatment mean 24-hour systolic BP) with treatment with a renin inhibitor, an angiotensin receptor blocker, and an angiotensin converting enzyme inhibitor, amongst REN-5312 T allele carriers. The study will have 80% power to detect 3 mm Hg differences in the primary end-point with treatment with the three RAS blockers amongst CC homozygotes.

Definitions of ITT and PP populations

Per protocol population; This population will correspond to all randomized patients who complete visits 1-9 without relevant deviations, which could effect the evaluation of mean 24 hour systolic BP. Efficacy will be determined from analyses of this population.

Intention to treat population; This population will correspond to all randomized patients who have taken at least one dose of study medication. Safety will be determined from analyses of this population.

18 CRITERIA AND PROCEDURES FOR DEALING WITH WITHDRAWALS FROM STUDY.

Withdrawal Criteria

Study treatments will be permanently discontinued for a participant for any of the following reasons;

- Onset of an adverse event which presents a risk to the patient, or which requires a prescription of a treatment which is prohibited by the protocol
- Renal function impairment with a decrease in creatinine clearance by >30% as assessed by Cockcroft's formula
    - Men = (140 - age) X weight (kg) / (0.814 X creatinine level (micromol/l)
    - Women = 0.85 X [(140 - age) X weight (kg) / (0.814 X creatinine level (micromol/l)]
- Increased potassium level > 5.5 mmol/l, confirmed in a repeated sample assayed within 7 days.
- Pregnancy
- Worsening of hypertension, defined as mean 24-hour ambulatory systolic BP > 160 mmHg.
- Non-medical reason (patient's personal decision to stop treatment).

Procedure For Dealing With Withdrawals

Patients withdrawn from the study will not be replaced. The sample size of the study allows for 10% withdrawal without impairing study power.

Patients who are withdrawn from the study will be reviewed by the study physician, and the most appropriate antihypertensive drug therapy will be prescribed. All such patients will have at least one follow-up out-patients visit in order to ensure good BP control without side effects.

Handling of data from subjects withdrawn from the study

Where patients are withdrawn prior to completion of all assessments of the week 8 visit, no comparison of responses are feasible, and no data from such patients will be included in efficacy analyses.

Where patients are withdrawn after the week 8 visit, efficacy data from such patients will be included in the efficacy analysis.

All available data from all patients will be included in all safety analyses.

19 CONTROL OF BIAS

Method of randomisation
All participants will then be allocated, in random order (stratified according to amlodipine usage or not), to 4 weeks of treatment with a renin inhibitor (aliskiren 300 mg daily), an angiotensin receptor blocker (candesartan 32 mg daily) and an angiotensin converting enzyme inhibitor (perindopril 8 mg daily). Randomisation will be fixed, balanced and organised through use of sealed envelopes.

Open versus Blinded Study

The design of this study is similar to a prospective randomized open therapy blinded end-points (PROBE) study. The primary end-point is the change from baseline in mean 24-hour systolic BP with each drug treatment. As ambulatory BP monitoring is fully automated, this is equivalent to an evaluator being blinded to treatment allocation.

20 EFFICACY ENDPOINTS:

Primary End-Point

The change from baseline in 24-hour systolic BP with each drug treatment.

Secondary End-Points

The change from baseline in 24-hour diastolic BP

The change from baseline in daytime systolic BP

The change from baseline in daytime diastolic BP

The change from baseline in night-time systolic BP

The change from baseline in night-time diastolic BP

The proportion of participants with controlled BP, defined as mean 24-hour systolic BP < 130mmHg and mean 24-hour diastolic BP < 80mmHg

The change from baseline in clinic systolic BP

The change from baseline in clinic diastolic BP

Data handling and Record Keeping

All data generated by the study will remain confidential and no report will contain any information that would allow an individual participant in the study to be identified. Paper clinical record forms (CRFs) will be used in this study. Data will be also be stored electronically using EXCEL (Microsoft EXCEL 97, Microsoft Corporation, Redmond, WA, USA) and ACCESS databases. Electronically stored data will be identified by a unique registration number, the participants' date of birth, and the participants' initials. All information relevant to the study will be stored for at least 15 years after the end of the study.

At the beginning of the study, an Investigator's Study File will be established at the study centre. The Investigator or appropriate designee is responsible for maintaining the study documents as specified in the ICH Guidelines for Good Clinical Practice Section 8 and as required by the applicable regulatory requirement(s). The Investigator/institution must take measures to prevent accidental or premature destruction of these documents.

21 SAFETY ENDPOINTS

The following safety measurements will be checked and recorded at each visit.

- Adverse events
- Assessment of concomitant diseases
- Clinic BP and HR
- Ambulatory BP monitoring
- Urea, electrolytes and creatinine estimation

22 ADMINISTRATIVE PROCEDURES

Ethics

The trial will not be initiated before the protocol, the informed consent form, the Patient Information Leaflet, details of the subject recruitment procedures and any other relevant study documentation have been reviewed and received approval / favourable opinion from the Irish Medicines Board (IMB) and the Beaumont Hospital Ethics (Medical Research) Committee (BEC). Should a substantial protocol amendment be made that requires IMB / BEC approval, the changes in the protocol will not be instituted until the amendment and the revised informed consent (if appropriate) has been reviewed and received approval / favourable response. A protocol amendment intended to eliminate an apparent immediate hazard to subjects may be implemented immediately providing that the appropriate authorities are notified as soon as possible and an approval is requested. Non substantial protocol amendments, for logistical or administrative changes only, may be implemented immediately.

The Principal Investigator or designee will report promptly to the IMB and BEC any new information that may adversely affect the safety of subjects or the conduct of the trial. Similarly, the Principal Investigator or designee will provide the ethics committee with a brief report of the outcome of the trial, if required

Informed Consent

If the patient meets all eligibility criteria and the Investigator confirms, the patient should be approached to obtain written informed consent. The background of the proposed study and the benefits and risks of the study should be explained to the patient. The patient must sign and personally date the consent form prior to enrollment. If the patient is unable to read, an impartial witness who has been present during the entire informed consent discussion must also sign and date the consent form. Failure to obtain signed informed consent renders the patient ineligible for the study. All enrolled patients will complete the appropriate consent form that has been approved by the Ethics Committee (EC) and the Sponsor. Copies of the signed informed consent shall be kept in the patient's medical records and study files. A copy of the informed consent form must be given to each patient enrolled in the study. Modifications to the Study Informed Consent must have written approval from the Sponsor and the EC prior to use.

Periodic Monitoring Procedures

An independent monitor will be contracted to visit the investigator periodically to verify the adherence to the protocol, the maintenance of the study-related records, and the completeness and accuracy of a proportion of CRF entries compared to source data. The investigator will co-operate with the monitor to ensure that any discrepancies that may be identified are resolved

Source Data and Records

Source data are all the information in original records and certified copies of original records of clinical findings, observations, or other activities in the study, which are necessary for the reconstruction and evaluation of the study. The investigator will permit study-related monitoring, audit(s), IEC/IRB review(s) and regulatory inspection(s), with a direct access to all the required source records. Further details will be specified in the CRF and Monitoring Guidelines.

Source records need to be preserved for the maximum period of time permitted by local requirements.

Insurance for Subjects

The Sponsor will provide the insurance conforming to national regulations for the subjects participating in this study.

Reporting

A final integrated clinical/statistical report will be prepared that is compliant with the ICH Harmonised Tripartite Guideline: Structure and content of clinical study reports (CPMP/ICH/137/95).

(to be printed on Beaumont Hospital headed paper)

Appendix 14.2

Patient Information Leaflet

Protocol Title:

Renin Genotype and Response to Renin Angiotensin System Blockade.

Principal Investigator:

Prof. Alice Stanton

Co-Investigators:

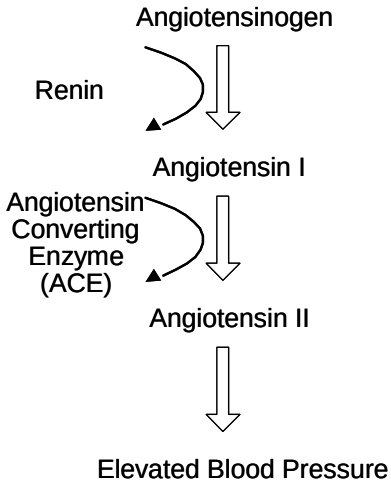
Dr. Brendan McAdam
Dr. Ursula Quinn

You are being invited to take part in a clinical research study which is being carried out at Beaum-ont Hospital. Before you decide whether or not you wish to take part, you should read the information provided below carefully and if you wish, please do discuss it with your family, friends or GP. Take time to ask questions – do not feel rushed or under any obligation to make a hasty judgement. You should clearly understand the risks and benefits of participating in this study so that you can make a decision that is right for you – this process is known as Informed Consent.

You are not obliged to take part in this study and failure to participate will have no effect on your future care. You may change your mind at any time (before the start of the study or even after you have commenced the study) for whatever reason without having to justify your decision and without any negative impact on the care you will receive from the medical staff.

WHY IS THIS STUDY BEING DONE?

The Renin Angiotensin System is important as it regulates blood pressure. If this system is overactive it can damage blood vessels, and lead on to heart attacks and strokes.



The Renin Angiotensin System can be explained by the above diagram and as follows; Renin converts Angiotensinogen to Angiotensin I, which is then converted to Angiotensin II by Angiotensin Converting enzyme (ACE). Angiotensin II stimulates receptors in arteries causing blood pressure to increase. This can lead to blood vessel damage and even heart attacks and stokes. Fortunately many drug treatments have been developed to block this system so as to control high blood pressure and to protect against heart attacks and strokes. These drugs include inhibitors of renin, ACE and angiotensin II receptors. Recent research by our group has suggested that an individual's genetic make-up can influence whether they get a good response from the various blocking drugs. In this study we are aiming to confirm our previous findings. If we do, the results of this study are likely to help doctors to prescribe the best blocker of the Renin Angiotensin System (the most effective with the least side effects) to individual patients in the future.

WHO IS ORGANISING AND FUNDING THIS STUDY?

This study is supported by a research grant from Enterprise Ireland. Dr Ursula Quinn, a Research Doctor who will in large part run this study, will include information from this study in her PhD thesis.

HOW WILL IT BE CARRIED OUT?

The study is due to commence in July 2008 and complete by June 2010. One hundred Beaumont Hospital patients will be studied.

WHAT WILL HAPPEN TO ME IF I AGREE TO TAKE PART?

If you decide to take part in this study, you will attend the hospital clinical research centre on nine occasions over a four month period. At four of these visits, you will only have a blood test and a 24-hour ambulatory blood pressure monitor will be fitted. The other five visits will include a range of assessments and will take approximately 90 minutes each.

On the first visit, a medical history will be taken and a physical examination will be performed. If you are suitable to join the study, you will be asked to stop any blood pressure lowering medication for one week. As elevated blood pressure does its damage over months and years, it has been shown that short periods off medications are not associated with any increase in risk.

One week later, both 24-hour ambulatory blood pressure and clinic blood pressure will be measured, and blood tests will be performed. Some of your blood will be stored for DNA extraction and genetic studies, and some for later biochemical studies. Patients whose blood pressure is suitable (not too high or too low) will be allocated (in random order) to 4 weeks of treatment with each of a renin inhibitor (aliskiren 300 mg daily), an angiotensin receptor blocker (candesartan 32 mg daily) and an angiotensin converting enzyme inhibitor (perindopril 10 mg daily). These are all standard blood pressure lowering treatments, at standard recommended doses. At the end of each 4 week treatment period (visits 3 to 5), clinic blood pressure and 24-hour ambulatory blood pressure will be measured, and standard safety blood tests will be performed.

If at your second visit, your blood pressure, when off all your usual blood pressure lowering treatments, is too high to enter the study, you will be treated with amlodipine 10mg daily for two weeks, and re-evaluated. If your blood pressure is now suitable, you will be continued on amlodipine, and can enter the study. Amlodipine is also a standard blood pressure lowering treatment, and has been shown to be effective and safe when combined with aliskiren, candesartan and perindopril.

At the end of the study your study records will be examined, and you will be prescribed the medication(s) which resulted in the best blood pressure control, so long as you did not have any side effects with this medication. If you are agreeable a summary letter will be sent to your general practitioner both at the beginning of the study and at the end.

WHAT ALTERNATIVE TREATMENTS ARE AVAILABLE TO ME?

Your blood pressure is elevated. Hence you will be better protected from suffering a heart attack or a stroke if you take blood pressure lowering medication. There is a wide range of blood pressure lowering therapies available. If you decide not to take part in this study, appropriate blood pressure lowering medication will be advised and prescribed for you.

BENEFITS:

Within this study, we will evaluate which blocker of the renin angiotensin system is best for you – which one controls your blood pressure best, without any important side effects. This means that your blood pressure is likely to be better controlled in the future, and that you will be better protected from heart attacks and strokes in years to come.

RISKS:

You will be asked to attend the hospital for extra visits, and to undergo a number of extra blood tests, which can be temporarily painful and can lead to arm bruising.

You will have a number of extra 24-hour ambulatory blood pressure monitors performed, and these can be inconvenient, and can cause either mild arm bruising or sleep disturbances.

Lastly, while all of the therapies within this study are commonly used standard blood pressure lowering drugs, they, like all medications, have the potential to cause side-effects. These are generally mild and infrequent, and usually resolve with stopping the treatment. The most common side-effects, occurring in 1% or greater of patients, who take any of the four drugs used within this study, are as follows;

Aliskiren: diarrhoea, dizziness due to too low blood pressure, and high potassium.

Candesartan: dizziness due to too low blood pressure, respiratory infections, headaches, vertigo and high potassium.

Perindopril: cough, dizziness due to too low blood pressure, high potassium, headaches, vision disturbance, tinnitus, vertigo, gastrointestinal upset, muscular cramps, rashes and itchiness.

Amlodipine: dizziness due to too low blood pressure, fatigue, headaches, palpitations, flushing, gastrointestinal upset, and ankle swelling.

Hence if you experience any of the above symptoms, or indeed any change in your wellbeing during the study, we would ask you to get in contact with the study physician.

If there is an accidental overdose of study medication, too low blood pressure is the most likely outcome. If this occurs you would be likely to feel very dizzy, particularly upon standing. If you experience this, please avoid standing, particularly in dangerous situations, and urgently contact a physician for further advice.

Very rarely, some of the drugs used in this study can cause an allergy-like reaction called angioedema. In the event of any signs suggesting an allergic reaction (in particular difficulties in breathing or swallowing, or swelling of the face, extremities, eyes, lips and/or tongue) you should discontinue the study treatment, and urgently contact a physician.

During pregnancy, particularly during the second and third trimesters, any of these drugs can cause injury to the developing fetus. Hence should any women become pregnant during the study, we would ask you to discontinue the study medication immediately, and to get in contact with the study physician.

In patients with diabetes who are taking antidiabetic medicines (insulin or oral hypoglycaemic agents), the addition of perindopril may lower blood sugar further. Hence more regular self-monitoring of blood sugars will be advised for diabetic patients throughout the study.

WHAT IF SOMETHING GOES WRONG AS A RESULT OF MY PARTICIPATION IN THIS STUDY?

Your doctors, nurses and Beaumont Hospital are covered by normal medical indemnity (insurance) schemes. This means that in the unlikely event of you being harmed or damaged though participation in this clinical trial, any reasonable claim you might make for compensation

can be covered by this insurance.

YOUR RESPONSIBILITIES AS A PARTICIPANT

We would ask you to contact the study doctor, Dr Ursula Quinn if you have any concerns about your health or any side effects, or if you commence any new medications during the study.

All female patients of child-bearing potential are advised to continue to use an adequate method of contraception. Should a pregnancy occur you must notify us immediately.

Please let us know if you change your mind about participation in the study, so that we can organise appropriate management of your blood pressure.

OUR RESPONSIBILITIES TO YOU AS INVESTIGATORS

It is our responsibility to advise you concerning the risks and benefits of participation in this study, and to care for you during and after the clinical trial. We will discuss with you any new information, which becomes available during the course of the study, if it could affect your willingness to continue to participate. If at any stage we think that it is not in your best interest to continue, we will arrange for you to discontinue participation in the study.

CONFIDENTIALITY ISSUES

The participants in this study have a right to privacy. All information that is collected during this study is strictly confidential. However, direct access to your medical records may be required by the sponsor, their representatives (such as study monitors and auditors) and representatives of the Ethics Committee or the government drug regulatory authorities, so as to verify the accuracy of the data collected during the study. No report will contain any information that would allow any individual participant to be identified.

Paper copies of your information will be stored in a locked filing cabinet. This information will be stored in Beaumont Hospital for 15 years, and then will be destroyed.

Any information stored on computer disk will not identify you by name, and will be password protected. This coded information may be stored indefinitely at either Beaumont Hospital or the Royal College of Surgeons in Ireland.

As previously mentioned we would like to retain portions of your blood samples, so that we have the possibility of further chemical and genetic studies. These samples would likewise be identified by a code rather than your name. These samples would remain indefinitely in storage in a laboratory at either Beaumont Hospital or the Royal College of Surgeons in Ireland, under the care of the Investigators of the study.

IF YOU REQUIRE FURTHER INFORMATION

If you have any further questions about the study, or if you wish to withdraw from the study you may do so without justifying your decision and your future treatment will not be effected.

For additional information now or any future time please contact:

Name Dr Ursula Quinn

Address Clinical Research Centre, Beaumont Hospital, Dublin 9.

Phone No 01 8092862

Appendix 14.3

(to be printed on Beaumont Hospital headed paper)

CONSENT FORM

Protocol Title: Renin Genotype and Response to Renin Angiotensin System Blockade.

Please tick the appropriate answer.

I confirm that I have read and understood the attached Patient Information Leaflet dated May 2008,, and that I have had ample opportunity to ask questions all of which have been satisfactorily answered. ☐ **Yes** ☐ **No**

I understand that my participation in this study is entirely **voluntary** and that I may withdraw at any time, without giving reason, and without this decision affecting my future treatment or medical care. ☐ **Yes** ☐ **No**

I understand that my records may be viewed by individuals with delegated authority from Prof Alice Stanton or Dr Brendan McAdam ☐ **Yes** ☐ **No**

I understand that my identity will remain confidential at all times. ☐ **Yes** ☐ **No**

I am aware of the potential risks of this research study. ☐ **Yes** ☐ **No**

I have been given a copy of the Patient Information Leaflet and this Consent form for my records. ☐ **Yes** ☐ **No**

I agree for correspondence to be sent to my GP in relation to my participation in this study

☐ **Yes** ☐ **No**

FUTURE USE OF ANONYMOUS DATA:

I agree that I will not restrict the use to which the results of this study may be put. I give my approval that unidentifiable data concerning my person may be stored or electronically processed for the purpose of scientific research and may be used in related or other studies in the future. (This would be subject to approval by an independent body, which safeguards the welfare and rights of people in biomedical research studies - the Beaumont Hospital Ethics (Medical Research) Committee.) ☐ **Yes** ☐ **No**

Patient _____ _____

Name in block capitals *Signature and dated*

To be completed by the Principal Investigator or her nominee.

I the undersigned, have taken the time to fully explained to the above patient the nature and purpose of this study in a manner that he/she could understand. I have explained the risks involved, as well as the possible benefits and have invited him/here to ask questions on any aspect of the study that concerned them.

_____ _____

_____ _____

Name in Block Capitals: *Signature:* *Qualification:* *Date:*

3 copies to be made: 1 for patient, 1 for PI and 1 for hospital records.

(to be printed on Beaumont Hospital headed paper)

GENETIC CONSENT FORM

Protocol Title: Renin Genotype and Response to Renin Angiotensin System Blockade.

Please tick appropriate boxes Yes/No

I am an **adult** taking part in this study. Yes □ No □

I have read the **Information Leaflet** about this research project, and have been given a copy to keep. The information has been fully explained to me and I have been able to ask questions. I understand why the research is being done and any risks involved. Yes □ No□

I agree to donate tissue sample (blood/DNA) for this research project. I understand that giving a sample for this research is **voluntary** and that I am free to withdraw my approval at any time without my medical treatment being affected. Yes □ No □

I give permission for research personnel to look at my medical records to obtain information. I have been assured that information about me will be kept confidential. Yes □ No □

Storage and Future uses of Biological Material
I give permission for my samples and information collected about me to be stored for possible future research related to this study (including DNA or genetic studies) but only if the research is approved by a Research Ethics Committee. Yes □ No□

Patient _____ _____

Name in block capitals *Signature and dated*

To be completed by the Principal Investigator or her nominee.

_____ _____ _____ _____

Name in Block Capitals: *Signature:* *Qualification:* *Date:*

3 copies to be made: 1 for patient, 1 for PI and 1 for hospital records.

Appendix 14.4
Ethics Committee Approval

Beaumont Hospital
Ethics (Medical Research) Committee

Chairperson: Professor Gerry McElvaney
Convenor: Professor Kieran Murphy

Administrator: Gillian Vale

7th August 2008

REC reference: 08/60 **EudraCT: 2008-003568-20** **Protocol No.: RGR 001**

Professor Alice Stanton
Associate Professor in
Molecular and Cellular Therapeutics
Royal College of Surgeons in Ireland
Dublin 2

Chief Investigator for Ireland: Professor Alice Stanton
RE: 08/60 – Prof. Alice Stanton – Renin Genotype and Response to Renin Angiotensin System Blockade

Dear Professor Stanton

The Recognised Ethics Committee reviewed the above application at its meeting held on the 27th June 2008. A Quorum was present at this meeting as outlined in S.I. 190 of 2004.

The Committee has given a favourable ethical opinion for the above clinical trial based on the application, protocol and supporting documentation (as listed in the attached document)

This study was given a favourable opinion on the 7th August 2008. This favourable opinion is extended to the site listed below only.

Chief Investigator & Principal Investigator	Site
Prof. Alice Stanton	Beaumont Hospital

There are no conditions attached to this favourable opinion.

Yours sincerely

Professor Gerry McElvaney
Chairperson
Ethics (Medical Research) Committee

Ethics (Medical Research) Committee - Beaumont Hospital
Notification of ERC/IRB Approval

Investigator: Professor Alice Stanton

REC reference: 08/60 **EudraCT: 2008-003568-20** **Protocol No.: RGR 001**

Protocol Title: Renin Genotype and Response to Renin Angiotensin System Blockade

Ethics Committee Meeting date: 27th June 2008

Final Approval Date: 7th August 2008

From: Ethics (Medical Research) Committee - Beaumont Hospital, Beaumont, Dublin

Documents Reviewed

Document and Date	Date Reviewed	Approved
Application Form, signed 5/6/08	7/8/08	Yes
Protocol RGR 001, V3, 29/7/08	7/8/08	Yes
Patient Information Leaflet, V3, 29/7/08	7/8/08	Yes
Consent Form, V3, 29/7/08	7/8/08	Yes
Genetic Consent Form, V3, 29/7/08	7/8/08	Yes
Letter of Invitation to Participants, V1, 5/6/08	7/8/08	Yes
GP Letter, V1, 5/6/08	7/8/08	Yes
Site Specific Assessment Form, signed A. Stanton, 5/6/08, M. Swords, 18/6/08	7/8/08	Yes
Financial Information, 5/6/08	7/8/08	Yes
SmPc: -		
Atacand (updated 23/8/07)	7/8/08	Yes
Rasilez (updated 2/11/07)	7/8/08	Yes
Istin (updated 25/9/07)	7/8/08	Yes
Conversyl Arginine (updated 1/5/07)	7/8/08	Yes

SSA: - signed A. Stanton, 5/6/08; Signed M. Swords, 18/6/08	**7/8/08**	**Yes**
Form of Indemnity	**N/A**	**N/A**
Irish Medicines Board Approval	**Pending**	**Pending**
CV, Prof. Alice Stanton, signed 5/6/08	**7/8/08**	**Noted**
Summary CV, Prof. Alice Stanton, signed 5/6/08	**7/8/08**	**Noted**

Professor Gerry McElvaney
ERC/IRB – Chairperson's Signature
Approval # 1, dated 7th August 2008

Appendix 14.5
Regulatory Approval

IRISH MEDICINES BOARD

8th August 2008

Professor Alice Stanton,
Royal College of Surgeon's Ireland,
RCSI Research Institute,
Dublin 2.

EUROPEAN COMMUNITIES (CLINICAL TRIALS ON MEDICINAL PRODUCTS FOR HUMAN USE) REGULATIONS, 2004

RE: CT number: CT 1355/2/1 - Aliskiren
Case number: 2051960
EudraCT number: 2008-003568-20
Protocol number: RGR
Title of trial: Renin Genotype and Response to Renin Angiotensin System Blockade

Dear Sirs,

The Irish Medicines Board has considered the application dated **2nd June 2008** seeking authorisation to conduct the above clinical trial.

On the basis of the evidence available, the application is acceptable.

Please note that the date of this letter is the date of authorisation of the trial.

Yours sincerely,

Sinéad Murphy
A person authorised in that behalf by the said Board

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