FINAL STUDY REPORT

Study Title	AMARYLIS Effect of the direct renin inhibitor aliskiren on the renal hemodynamics and metabolic parameters in patients with pre - diabetes
Study Code	ALI-FRA-0030-I
EudraCT-Number	2008-008287-28
Name of Study Drug	Aliskiren (Rasilez ®)
Indication	Prediabetes
Study Phase	Phase IIIb, prospective, open-label, single center study
Study Duration	First patient in (FPI): March 3rd, 2012 Last patient out (LPO): June 27th, 2013
Version	1.0
Sponsors:	Medical Faculty (University Erlangen) Prof. Roland Schmieder (representative of the sponsor) Maximiliansplatz 2, 91054 Erlangen

Approval Signatures

This final study report for the AMARYLIS study ALI-FRA-0030-I, EudraCT Number 2008-008287-28 in the present version V 1.0 dated 16th May 2014 is deemed binding by the signatures:

Prof. Dr. med. Roland Schmieder Leading investigator (LKP) Dept. of Nephrology and Hypertension, Head of Clinical Research Center Ulmenweg 18, 91054 Erlangen	Date: 19.05.2014 Signature
Priv.-Doz. Dr. med. Christian Ott Study Statistician Dept. of Nephrology and Hypertension Clinical Research Center, Ulmenweg 18, 91054 Erlangen	Date 19.05.2014 Signature
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STUDY SYNOPSIS

AMARYLIS Study **Effect of the direct renin inhibitor aliskiren on the renal hemodynamics and metabolic parameters in patients with pre - diabetes**	
Objectives	Primary objective: To investigate the effect of aliskiren (1. week 150mg/d, 2. to 5. week 300mg/d) on parameters of renal hemodynamics in patients with pre – diabetes and hypertension without evident renal disease. Parameters are: • Glomerular filtration rate (GFR) • Renal plasma flow (RPF) • Filtration fraction (FF) Secondary objectives • to investigate the effect of aliskiren (1. week 150mg/d, 2. to 5. week 300mg/d) on metabolic and inflammatory parameters (HbA1c, high sensitive CRP, adiponectin) in patients with pre – diabetes and hypertension without evident renal disease. Safety evaluation Monitoring and recording of all adverse and serious adverse events, regular monitoring of hematology, blood chemistry and urine values, regular measurement of vital signs and the performance of physical examinations
Study design	Phase IIIb, prospective, open, single center study
Patient Population	At least 50 subjects were to be included in order to obtain at least 30 fully evaluable subjects. Subjects were recruited from our University Outpatient Clinic, referring physicians and advertisements in local newspapers.

Inclusion criteria	• Pre - diabetes mellitus defined by fasting glucose ≥126 mg/dl or HbA1c 5.7-6.4% or impaired glucose tolerance • Age of 18 - 70 years • Blood pressure (mmHg) > 130 systolic or 85 diastolic
Exclusion criteria	• Manifest type 1 or 2 diabetes mellitus • Micro – or macroalbuminuria • Renal insufficiency (eGFR < 60 ml/min/1.73m²) • Secondary arterial hypertension • Body mass index >40 kg/m² • Known liver function test >2 times upper limit of normal • Pregnant or breast-feeding patients • Current treatment with an ACE inhibitor, AT1 – blocker or direct renin inhibitor or with more than 2 antihypertensives • Uncontrolled hypertension • Myocardial infarction or stroke < 6 months prior to screening visit (visit 1) • Patients being treated for severe gastrointestinal disease • Heart failure NYHA III-IV • Abuse of medications or alcohol • Present malignant disease • Individuals at risk for poor protocol or medication compliance • Women without adequate birth control • Known non-tolerance of direct renin inhibitors

Discontinuation of study participation	• adverse event(s) • abnormal laboratory value(s) • abnormal test procedure result(s) • unsatisfactory therapeutic effect • subject's condition no longer required study treatment • protocol violation • subject withdrew consent • lost to follow-up • administrative problems • death
Primary Endpoints	• Glomerular filtration rate (GFR) • Renal plasma flow (RPF) • Filtration fraction (FF) after 5 weeks of aliskiren therapy (1. week 150mg/d, 2.-5. week 300m/d)
Secondary Endpoints	• HbA1c • High sensitive CRP • Adiponectin after 5 weeks therapy with aliskiren (1. week 150mg/d, 2.-5. week 300mg/d)
Sample size calculation	10-15% estimated GFR and RPF reduction by therapy with aliskiren, 30 patients + 20 drop outs, α=0.05, β=0.80, SD = 9 %
Study visits	<u>Screening/wash-out phase of</u> • 4 weeks, if pretreated with any RAS blocking agent <u>Visit 3</u> • Measure of renal hemodynamics while stressed with i.v. glucose infusion • Start with aliskiren 150mg/die <u>Visit 4 – Safety visit after 5 ± 2 days</u> • Serum electrolytes, creatinine, urea • Clinical examination

	• Blood pressure • Start with aliskiren 300mg/d (downtitration to 150mg/d possible in case of systolic blood pressure < 120/70 mmHg or orthostatic symptoms) <u>Visit 6</u> • Physical examination • Blood pressure • Renal hemodynamics during glucose stress
Schedule	First patient in (FPI): March 03rd, 2012 Last patient out (LPO): June 27th, 2013 Data base lock: December 2013
Investigational product	• Aliskiren 150/300 mg film-coated tablet
Concomitant medication	• Stable therapy with respect to statins, antihypertensive medication, and antiplatelet agents was allowed • No additional RAAS blocking medication was allowed during the entire study

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LIST OF ABBREVIATIONS

ABPM	Ambulatory Blood Pressure Monitoring
ACE	Angiotensin Converting Enzyme
AE	Adverse Event
ALAT	Alanin-Aminotransferase (also: SGPT)
AMG	Arzneimittelgesetz (German Drug Law)
ASAT	Aspartat-Aminotransferase (also: SGOT)
AT1	Angiotensin Type-1
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
BMI	Body mass index
CRC	Clinical Research Center
CRF	Case Report Form
CRO	Clinical Research Organization
ECG	Electrocardiogram
(e)GFR	(estimated) Glomerular Filtration Rate
EU	European Union
FF	Filtration Fraction
FPI	First Patient In
FSR	Final Study Report
GGT	Gamma-Glutamyltranferase (also: γ-GT)
HbA1c	glycosylated hemoglobin
HDL	High Density Lipoprotein
HOMA	Homeostasis Model Assessment
hsCRP	high-sensitive C-reactive Protein
ICF	Informed Consent Form
IEC	Independent Ethics Committee
IIT	Investigator Initiated Trial
IN	Inulin
IRB	Institutional Review Board
ITT	Intention-to-treat
LDL	Low Density Lipoprotein
LKP	Leiter der klinischen Prüfung (also: Leading investigator)
LPO	Last Patient Out

NO	Nitric Oxide
OGTT	Oral Glucose Tolerance Test
PAH	Para-Amino-Hippur-Acid
PP	Per protocol
RA(A)S	Renin Anagiotensin Aldosterone System
RBC	Red Blood Cell Count
RBF	Renal Blood Flow
RPF	Renal Plasma Flow
SADR	Suspected Adverse Drug Reaction
SAE	Serious Adverse Event
SAF	Safety population
SAS	Statistical Analysis Software
SCR	Screening
SFU	Safety Follow-up
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
UACR	Urine Albumin to Creatinine Ratio
UV	Unscheduled Visit
WBC	White Blood Cell Count
WHO	World Health Organisation
ZV	Zwischenvisite

1 Rationale

Nearly 18 Million adult people suffer from type 2 diabetes mellitus in the U.S., further 16 million are estimated suffering from impaired glucose tolerance.

Type 2 diabetes is the leading course of terminal renal insufficiency [1] and worldwide increasing [2]. Patients with diabetic nephropathy are at high risk of cardiovascular events [3]. Patients with only prediabetes are at increased risk for cardiovascular and renal complications. Strategies for prevention and inhibition of progression of prediabetes to a manifest type 2 diabetes is important and of major prognostic relevance.

Activation of the renin angiotensin system (RAAS) seems being the pathophysiological central pathway in the development of diabetic nephropathy. Research suggests a deceleration of diabetes progression by inhibition of the RAAS. AT1- receptor blocker have shown to inhibit progression of diabetic nephropathy [4]. ACE-inhibitors, AT1-receptor blocker and direct renin inhibitors are available drug classes known to be effective in inhibiting the RAAS.

Aliskiren is a direct renin inhibitor that decreases plasma renin activity and inhibits conversion of angiotensinogen to angiotensin I. Aliskiren is approved as antihypertensive medication. Additional effects of aliskiren on renal hemodynamic have not been investigated yet.

The present study investigated the effect of aliskiren on renal hemdodynamic as well as on metabolic and inflammatory parameters in prediabetic patients. The objective was to analyse early changes of renal hemodynamics during an acute glucose provocation with and without treatment of aliskiren.

2 Ethics

2.1 Independent Ethics Committee

The study was approved by the Ethics Committee of the University of Erlangen (IRB/IEC) on 13-11-2011.

2.2 Ethical Conduct of the Study

The study was conducted in accordance with the Declaration of Helsinki.
The confidentiality of patient data was always guaranteed in accordance with the provisions of the EU Directive 95/46/EC and the national data protection and privacy legislations. The identities of the patients were encrypted and only authorized personnel had access to identifying data. The investigating physicians maintained an identification list.
The identities of the patients were pseudonymized. The responsibility for information from the personal medical files lay with the physician.

2.3 Subject Information and Consent

Before a patient could be included in this study, the patient had to have given his/her consent to participation in the study and to the recording, forwarding and viewing of his/her data after the physician had previously provided an adequate explanation of the objectives of the study to the patient. The patients had the opportunity to ask questions and receive satisfactory answers. The patients were also given sufficient time to decide whether to participate in this study or not. The written consent of the patient had to be obtained before he/she took part in the study and had to be documented in the medical file of the patient. The ICF had to be signed and personally dated by the patient and the physician who conducted the patient information discussion. One original copy of the signed ICF was kept by the physician, and the patient was handed a second original of the signed ICF. The date on which the ICF was signed had to be documented in the CRF.

In collecting and documenting the signing of informed consent, the participating physician had to comply with the applicable statutory provisions, the relevant local regulations and the ethical principles of the Declaration of Helsinki. The declaration of consent as well as any changes were approved by the ethics committee before they were given to potential participants.

3 Investigators and study administrative structure

3.1 Investigators

Participating sub-investigators were selected by the principal investigator Prof Roland Schmieder, Head of Clinical Research Centre (CRC), Department of Nephrology and Hypertension, University Hospital Erlangen, Ulmenweg 18, 91054 Erlangen. He acted as "Leading investigator" (LKP) according to German Drug Law (AMG).

Sub-investigators:

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Dr. med. Stephanie Schmidt
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3.2 Study Administration

The specific information related to the various objectives of this study was documented for each patient in a paper questionnaire (case report form, CRF).

In the paper documentation, each form was marked with a number and code for the study; the relevant patient serial number was entered on the form.

The participating physicians were responsible for ensuring that all patient data collected for this study were entered correctly into the CRFs. The CRFs were collected by the CRC for further processing. All incoming CRFs were registered; afterwards all data from the CRFs were entered in the project-specific database (double data entry) by the CRC personnel.

The CRC was responsible for preparing a data management plan, a data validation plan, the database, and for data entry including entry controls.

Data management was performed by Ingrid Fleischmann under the supervision of the CRC.

3.3 Sponsor

The sponsor of this investigator initiated trial (IIT) was the Medical faculty (represented by the dean) of the University of Erlangen-Nürnberg, delegated to Prof. Dr. Roland Schmieder, Head of CRC, Department of Nephrology and Hypertension, University Hospital Erlangen, Ulmenweg 18, 91054 Erlangen.

3.4 CRO

The clinical research organization (CRO) IPPMed Institut für Pharmakologie und Präventive Medizin GmbH, Osterstr. 15, 49661 Cloppenburg was involved in monitoring the study sites. On-site visits were made before trial start, four times during the trial and at the end (close-out visit). The monitor had the responsibility of reviewing the ongoing trial with the investigator to verify adherence to the protocol and to deal with any problems if and when they arose. The confidentiality of trial documents was maintained at all times.

3.5 Laboratory values

Laboratory evaluations for this study were carried out by the accredited central lab of the University Hospital Erlangen and by the laboratory of the Department of Nephrology and Hypertension, University of Erlangen-Nürnberg.

4 Objectives of the study

4.1 Primary objective

- To investigate the effect of aliskiren (1. week 150mg/d, 2. to 5. week 300mg/d) on parameters of renal hemodynamics in patients with pre –diabetes and hypertension without evident renal disease was considered the primary objective. The investigated parameters were:
  - Glomerular filtration rate (GFR)
  - Renal plasma flow (RPF)
  - Filtration fraction (FF)

4.2 Secondary objectives

- To investigate the effect of aliskiren (1. week 150mg/d, 2. to 5. week 300mg/d) on metabolic and inflammatory parameters in patients with pre – diabetes and hypertension without evident renal disease. The investigated parameters were:
  - HbA1c
  - High sensitive CRP
  - Adiponectin

5 Study design

5.1 Overall study design

The study was a phase IIIb, prospective, open single center study.
After a 4 weeks run-in/wash-out phase for patients with any RAS blocking agent therapy patients received aliskiren 150 mg/d for one week, followed by aliskiren 300mg/d for another 4 weeks. For RAS blocker naïve patients no washout phase was needed.
Note: At beginning of run-in/wash-out phase, patients on RAS blocking agent could be switched to amlodipine 5 – 10mg/day, which were kept constant throughout the study.

5.2 Study population

Subjects were recruited from the University Outpatient Clinic, referring physicians (family practioner, specialists in general or internal medicine), and advertisements in local newspapers. After a first contact by phone eligible patients were invited to an interview and selected using a standardized questionnaire. Eligible patients for the study were scheduled for a screening visit.

5.2.1 Inclusion and exclusion criteria

5.2.1.1 Inclusion criteria

- Pre - diabetes mellitus defined by fasting glucose ≥126 mg/dl or HbA1c 5.7-6.4% or impaired glucose tolerance
- Age of 18 - 70 years
- Blood pressure (mmHg) > 130 systolic or 85 diastolic

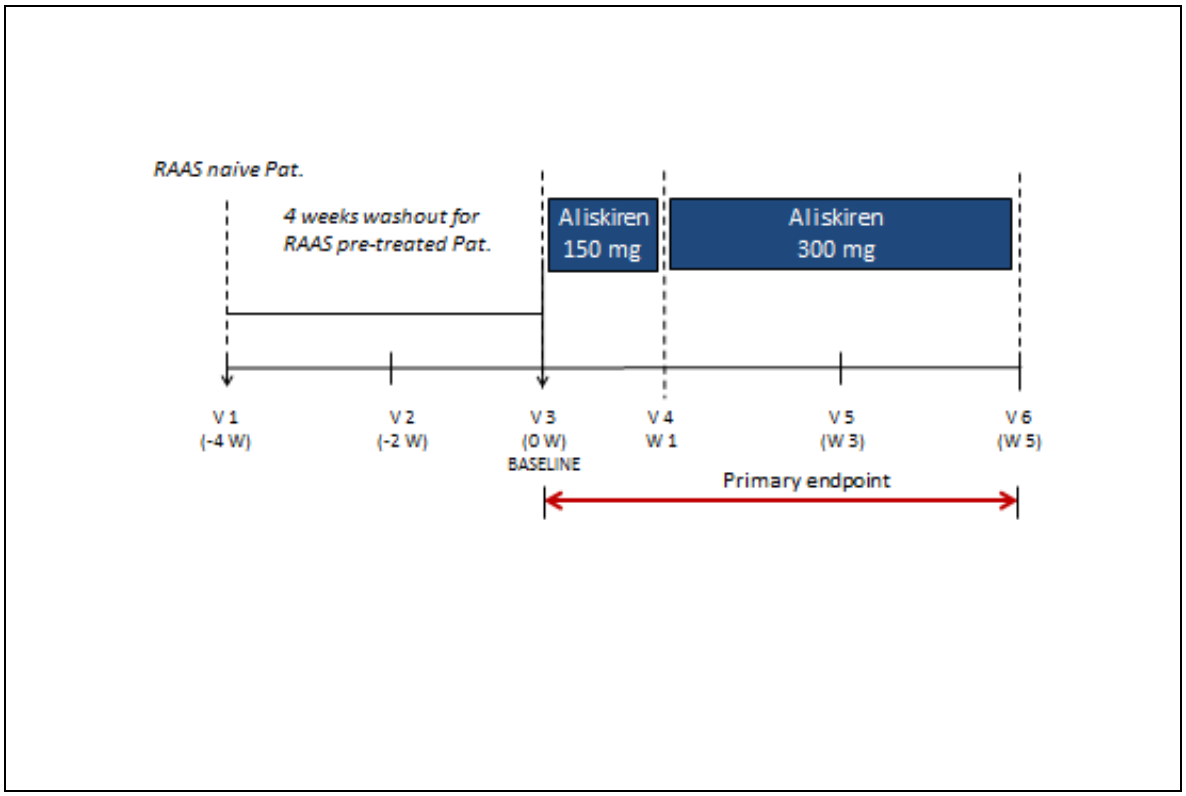
5.2.1.2 Exclusion criteria

- Manifest type 1 or 2 diabetes mellitus
- Micro – or macroalbuminuria
- Renal insufficiency (eGFR < 60 ml/min/1.73m²)
- Secondary arterial hypertension
- Body mass index >40 kg/m²
- Known liver function test >2 times upper limit of normal
- Pregnant or breast-feeding patients
- Current treatment with an ACE inhibitor, AT1 – blocker or direct renin inhibitor or with more than 2 antihypertensives
- Uncontrolled hypertension
- Myocardial infarction or stroke < 6 months prior to screening visit (visit 1)
- Patients being treated for severe gastrointestinal disease
- Heart failure NYHA III-IV
- Abuse of medications or alcohol
- Present malignant disease

- Individuals at risk for poor protocol or medication compliance
- Women without adequate birth control
- Known non-tolerance of direct renin inhibitors

5.3 Study flowchart

Figure 5-1 Trial Flow Chart



5.4 Visit schedule

Figure 5-2 Visit Schedule

Week (Total Time: at maximum 36 Days)	-4	-2	0	0	1	3	5	5
Visit No.	**1**	**2***	**ZV**	**3**	**4**	**5**	**ZV**	**6**
Vital Signs	X	X		X	X	X		X
Adverse Events		X		X	X	X		X
Concomitant Medication	X	X		X	X	X		X
Height/Weight / BMI / Hip /	X			X				X
Physical Examination	X	X		X	X	X		X
Informed Consent	X							
Inclusion / Exclusion Criteria	X			X				
Medical History	X							
Demographic Data	X							
ECG (12-lead)	X							
24 h-RR disposal			X				X	
Blood- and Urine Sample								
• OGTT	X							
• Safety Laboratory	X			X	X	X		X
• Urine - Analysis	X			X				X
• Urine - Pregnancy Test[#]	X			X				X
• First Morning Urine and Spot Urine				X				X
Assessment of renal hemodynamics and function								
• CLEARANCE / CLAMP Test				X				X
Medication Dispense								
• Aliskiren				X	X			
Medication Retrieval								
• Aliskiren					X			X
Compliance Check					X			X

* Patients not pre-treated with RAAS antihypertensive medication do not have a wash-out phase and skip Visit 2;
Only patients who require a 4 week wash-out period start with V1 followed by visit 2 and visit 3

Females only (if applicable)

5.4.1 Visit 1 (week-4)

The following activities and/or assessments were performed at/during screening (Visit 1):

- Recording of demographic data and medical history including any concomitant medication
- A physical examination including measurement of waist and hip circumference, height and weight
- Measurement of vital signs (i.e. casual blood pressure and heart rate: blood pressure and heart rate, measured after 5 minutes of rest in a sitting position with an appropriate cuff size with an automatic oscillometric device fulfilling quality assurance criteria; mean of 3 measurements were calculated).
- A 12-lead routine-ECG recorded at rest in a supine position
- An oral glucose tolerance test
- Fasting blood samples drawn for:

Safety Laboratory Markers:

- Biochemistry incl. urea, creatinine, uric acid, sodium, potassium, calcium, γ-GT, total bilirubin, ALAT/SGPT, ASAT/SGOT, alkaline phosphatase, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, fasting glucose, HbA1c, hs-CRP
- Hematology incl. hemoglobin, hematocrit, red blood cell count, platelet count, white blood cell count
- Urinalysis:
    - spot urine: creatinine, albumin, sodium;
    - Urinanalysis (dipstick): incl. protein, glucose, blood, white blood cells, pH, ketonuria, nitrite
    - Micraltest for protein
- Urine pregnancy test (applicable females only).

At the end of the visit all eligible patients started to wash-out RAAS antihypertensive medication during the 4 weeks washout phase.
Patients who required a 4 week wash-out period had an additional Visit after 2 weeks.
Patients who were not pretreated with RAAS antihypertensive medication did not have

a wash-out phase and skipped Visit 2 (i.e. V3 followed after V1).

5.4.2 Visit 2 (week -2)

Visit 2 was scheduled for pretreated patients who required a 4 week wash-out period,
This visit included vital signs (i.e. casual blood pressure and heart rate), physical examination, checking concomitant medication and assessment of adverse events.

ZV

- Dispensing of 3 urine collecting boxes including instructions for sampling handed out
- Dispensing of 24 hours ABPM device including instructions for sampling handed out

5.4.3 Visit 3 (Randomization, Baseline Visit, week 0)

Visit 3 took place 2 weeks after Visit 2 (for pretreated patients who required a 4 week wash-out period) or after Visit 1 for patients not pretreated with RAAS antihypertensive medication, respectively:

The following activities and/or assessments were performed at Visit 3:

- Receiving box for first morning spot urine collection
- Receiving box for 24-h urine sample
- Rechecking of in- and exclusion criteria.
- Assessment of adverse events
- A physical examination including measurement of waist and hip circumference, height and weight
- Measurement of vital signs (i.e. casual blood pressure and heart rate; blood pressure and heart rate were measured after 5 minutes of rest in a sitting position with an appropriate cuff size with an automatic oscillometric device fulfilling quality assurance criteria; mean of 3 measurements was calculated).
- Fasting blood samples were drawn for:

Safety Laboratory Markers:

- Biochemistry incl. urea, creatinine, uric acid, sodium, potassium,

calcium, γ-GT, total bilirubin, ALAT/SGPT, ASAT/SGOT, alkaline phosphatase, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, fasting glucose, HbA1c, hs-CRP

- Hematology incl. hemoglobulin, hematocrit, red blood cell count, platelet count, white blood cell count
- Urinalysis:
    - 24-h urine: sodium, potassium, creatinine, urea
    - spot urine: creatinine, albumin, sodium;
    - Urinanalysis (dipstick): incl. protein, glucose, blood, white blood cells, pH, ketonuria, nitrite
    - Micraltest for protein
- Urine pregnancy test (applicable females only).

- Study medication (aliskiren 150 mg) was dispensed to the patient

5.4.4 Visit 4 and visit 5 (week +1 and week +3)

These visits included physical examination, safety laboratory markers - including biochemistry, hematology and urinalysis - and vital signs (i.e. casual blood pressure and heart rate), checking concomitant medication and assessment of adverse events.
Following activities were done additional at visit 4:

- Compliance check for study medication (aliskiren 150 mg)
- Study medication (aliskiren 300 mg) was dispensed to the patient.

ZV

- Dispensing of 3 urine collecting boxes including instructions for sampling handed out
- Dispensing of 24 hours ABPM device including instructions for sampling handed out

5.4.5 Visit 6 (week +5)

5 weeks after Visit 3, Visit 6 took place.

The following activities and/or assessments were performed at Visit 6:

- Receiving box for first morning spot urine collection
- Receiving box for 24-h urine sample
- Assessment of adverse events
- A physical examination including measurement of waist and hip circumference, height and weight
- Assessment of vital signs (i.e. casual blood pressure and heart rate; blood pressure and heart rate were measured by after 5 minutes of rest in a sitting position with an appropriate cuff size with an automatic oscillometric device fulfilling quality assurance criteria; mean of 3 measurements were calculated), physical exam and body weight.
- Fasting blood samples were drawn for:

Safety Laboratory Markers:

- Biochemistry incl. urea, creatinine, uric acid, sodium, potassium, calcium, γ-GT, total bilirubin, ALAT/SGPT, ASAT/SGOT, alkaline phosphatase, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, fasting glucose, HbA1c, hs-CRP
- Hematology incl. hemoglobin, hematocrit, red blood cell count, platelet count, white blood cell count
- Urinalysis:
    - 24-h urine: sodium, potassium, creatinine, urea
    - spot urine: creatinine, albumin, sodium;
    - Urinanalysis (dipstick): incl. protein, glucose, blood, white blood cells, pH, ketonuria, nitrite
    - Micraltest for protein
- Urine pregnancy test (applicable females only).

5.4.6 Premature termination visit

In case of withdrawal from the study, subjects were asked to report to the clinical center for a Premature Termination visit.

5.4.7 SFU visit (Safety Follow-up)

Only in case of ongoing AE and/or at the investigator's discretion a SFU visit was to be performed. Relevant safety assessments: safety lab, urinalysis, vital signs, physical examination, 12-lead ECG, AE assessment, checking concomitant medication.

5.4.8 UV (Unscheduled Visit)

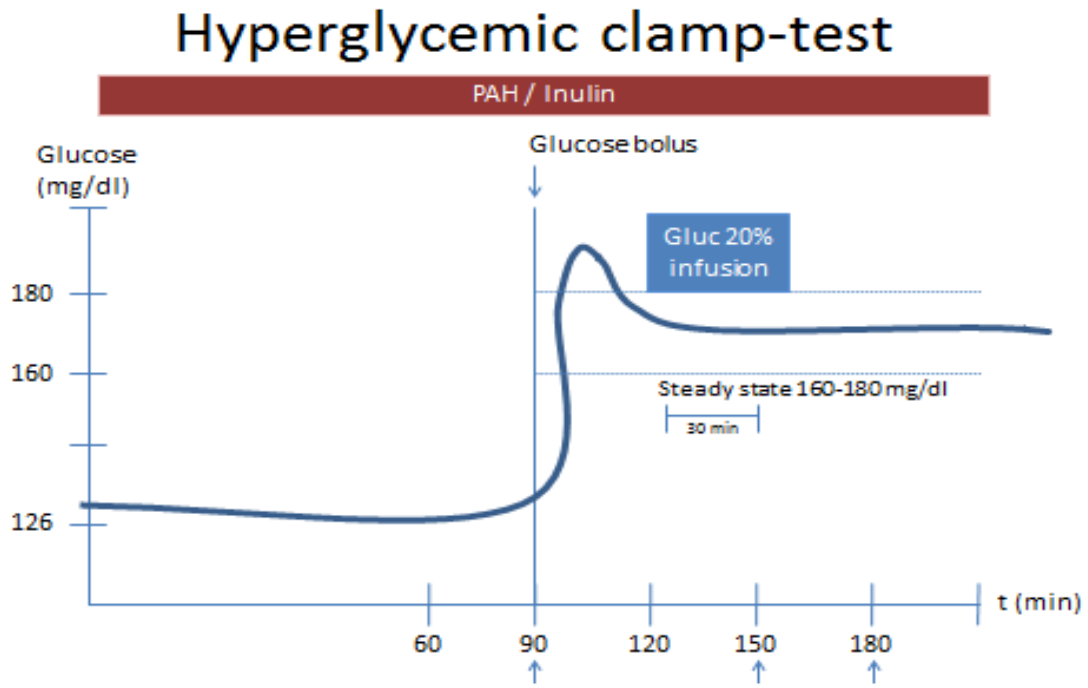
In case subjects were seen at additional times other than the regular scheduled study visits, if deemed necessary by the Investigator, the same investigations as for the SFU visit were performed and documented accordingly.

5.5 Assessment of renal function

5.5.1 Glucose – Insulin - Clamp

An intravenous glucose bolus (glucose 20%) was given via an intravenous access. After that a titrated infusion of 20% glucose was applied to increase blood glucose concentration up to the target of 160-180 mg/dl. The technique followed the instructions by De Fronzo et al [5].

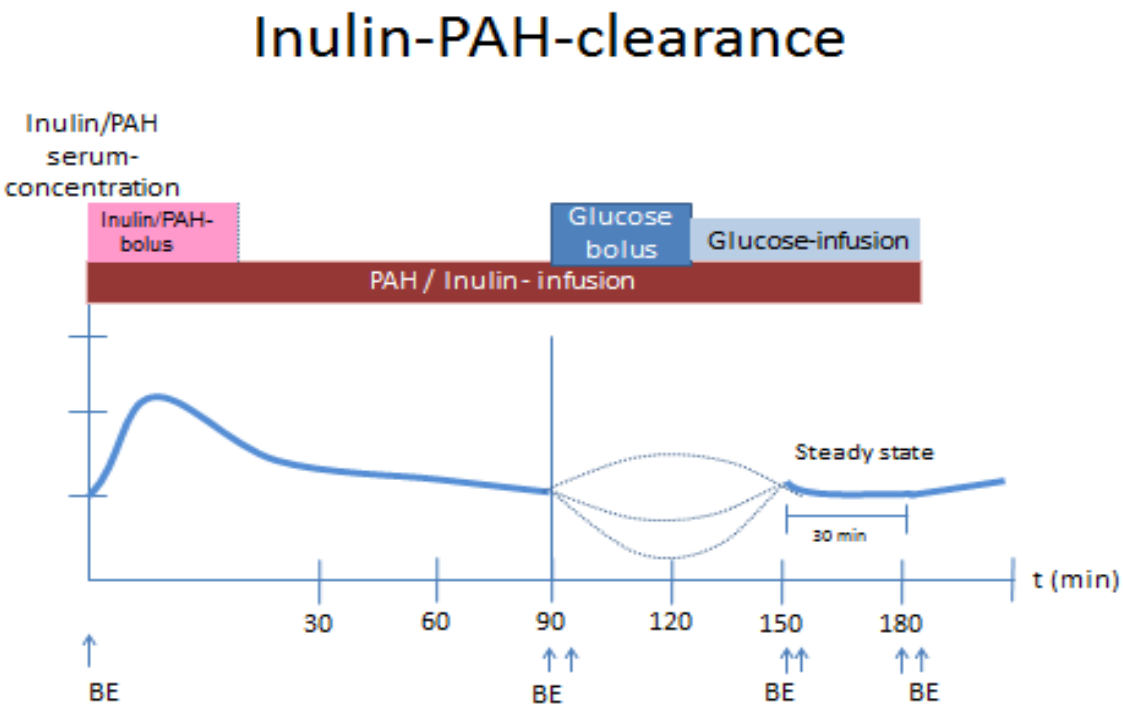
Figure 5-3 Hyperglycemic clamp-test



5.5.2 Measurement of glomerular filtration rate and renal plasma flow

GFR was determined by measurement of inulin (IN) clearance. RPF was determined by para-amino-hippur-acid (PAH)-Clearance. Both testing substances were dose adapted to body weight and applied by an intravenous access. After 1.5 hours a steady state was reached and blood samples taken for measurement of both test substance blood concentrations. IN-and PAH-clearance was calculated using the measurement principles of the constant-infusion technique without urine collection [6].

Figure 5-4 Inulin-PAH-clearance



Non-invasive hemodynamic parameters (blood pressure, heart rate) are measured every 10 minutes, blood glucose levels every 2 minutes.

5.6 Safety assessments

Safety assessments comprised monitoring and recording of all adverse events (AE) and serious adverse events (SAE), the regular monitoring of hematology, blood chemistry and urine values, regular measurement of vital signs and the performance of physical examinations.

5.6.1 Definition of an AE

An AE is any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (e.g. abnormal laboratory finding), symptom, or disease temporally associated with

the use of a medicinal product, whether or not considered related to the medicinal product. In clinical studies, an AE can include an undesirable medical occurrence at any time, even if no study treatment has been administered.

5.6.2 Definition of an SAE

A SAE is an AE that fulfills 1 or more of the following criteria:

- Results in death
- Is immediately life-threatening
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital abnormality or birth defect
- Is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above

In addition, the following events (regardless of whether or not they meet any of the seriousness criteria above) were collected as SAEs:

- Cancer
- Drug dependency/abuse
- Suspected transmission of an infectious agent

5.6.3 Reporting of AE

Each AE had to be reported on an Adverse Event Case Report Form. As far as possible, each AE also had to be described by: 1. its duration (start and end dates), 2. its severity grade (mild, moderate, severe) 3. its relationship to the study drug (suspected / not suspected) 4. the action(s) taken. Examples of the severity grade, relationship to study treatment and actions taken, as presented in the case report form, are provided below. The severity grade of an adverse event provides a qualitative assessment of the extent or intensity of an AE, as determined by the investigator or as reported by the subject. The severity grade does not reflect the clinical seriousness of the event, only the degree or extent of the affliction or occurrence (e.g. severe nausea, mild seizure), and does not reflect the relationship to study drug.

Severity grade for an AE

1 = Mild	The degree/extent/intensity of the event is mild.

2 = Moderate	The degree/extent/intensity of the event is moderate.
3 = Severe	The degree/extent/intensity of the event is severe.

The relationship between the administration of study drug and the occurrence of the AE is described as belonging to one of only 2 categories, either suspected by the investigator or not suspected by the investigator.

Relationship of adverse events to study drug

Not suspected	The temporal relationship of the clinical event to study drug administration makes a causal relationship unlikely, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.
Suspected	The temporal relationship of the clinical event to study drug administration makes a causal relationship possible, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

5.6.4 Reporting of SAEs

All SAEs as well as reports of pregnancy, lack of efficacy, medication error or overdosing irrespective of whether these were associated with an adverse event had to be reported to the Pharmacovigilance Department of Novartis Pharma GmbH by the principal investigator within 24 hours of becoming aware of the event. Furthermore, the principal investigator had to notify the Competent German Health Authority and the Ethics Committee of the University of Erlangen-Nürnberg respectively with respect to any SUSAR (suspected unexpected serious adverse reaction) that occurred within the scope of the analysis.

5.6.5 Laboratory evaluations

During the study blood samples were drawn at pre-specified visits (V1, V3, V4, V5, V6) to determine the following parameters:

SAFETY LABORATORY MARKERS:

- Biochemistry incl. urea, creatinine, uric acid, sodium, potassium, calcium, γ-GT, total bilirubin, ALAT/SGPT, ASAT/SGOT, alkaline phosphatase, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, fasting glucose, HbA1c, hs-CRP
- Hematology incl. hemoglobulin, hematocrit, red blood cell count, platelet count, white blood cell count
- Urinalysis:
    - 24-h urine: sodium, potassium, creatinine, urea (V3 and V6)
    - spot urine: creatinine, albumin, sodium;
    - Urinanalysis (dipstick): incl. protein, glucose, blood, white blood cells, pH, ketonuria, nitrite
    - Micraltest for protein
- Urine pregnancy test (applicable females only) (V1, V3, and V6)

5.6.6 Vital signs

5.6.6.1 BLOOD PRESSURE MEASUREMENTS

According to the recommendations of the WHO (mean from 3 measurements in a sitting position after 5 min rest).

5.6.6.2 MEASUREMENT OF HEART RATE

In parallel with blood pressure measurement.

5.6.7 Physical examination

Physical examination was performed prior to study inclusion, at pre-specified visits and – when indicated – during the study course. Significant findings that were present prior to the start of study drug were included in the Relevant Medical History/ Current Medical Conditions Case Report Form. Significant findings made after the start of study drug which met the definition of an AE were recorded on the Adverse Event Case Report Form.

5.7 Treatment discontinuation or interruption

It was documented whether or not each patient completed the clinical study. If for any patient either study treatment or follow up were discontinued the reason was recorded.

6 Treatment

Eligible patients entered:

- a 4 week run-in/wash-out phase, if pretreated with RAAS antihypertensive agent

After the run-in/wash-out phase, patients started therapy with aliskiren 150mg/d (Rasilez™) for 1 week, followed by 300 mg/d for 4 weeks. The duration of the treatment period was 5 weeks. Hence, the total study duration for each participant was up to 9 weeks.

6.1 Investigational therapy and reference therapy

Dosage form and route of administration for the investigational product:

- aliskiren (Rasilez™) 150 mg film coated tablet
- aliskiren (Rasilez™) 300 mg film coated tablet

All tablets were for oral administration.

6.1.1 Pharmaceutical Formulation/s

Name:	Aliskiren (Rasilez™)
Dosage form:	Film coated tablet
Strength:	150 / 300 mg
Pharmaceutical Manufacturer:	Novartis Pharma GmbH 90429 Nürnberg, Germany Tel: +49 (0) 911 -273 – 0

6.1.2 Labelling and Packaging

Aliskiren 150 / 300 mg was obtained as regular merchandise as (Rasilez™) 150 / 300 mg.

6.1.3 Dispensing Procedures and Storage Conditions

Aliskiren (Rasilez™) was bought from Novartis Pharma GmbH by the University Hospital Erlangen. The study drug was taken from the pharmacy of the University Hospital Erlangen to the clinical research unit of the Medical Department 4, University of Erlangen-Nürnberg (Head of the clinical research unit is the principal investigator). Upon receipt of the study medication supplies, an inventory was performed and a drug receipt log filled out and signed by the person accepting the shipment (study drug). Any damaged or unusable study drug in a given shipment (active drug or comparator) was documented in the study files. The principal investigator and sponsor was informed of any damaged or unusable study treatments that were supplied to the investigator's site. Regular study drug reconciliation was performed to document drug assigned, drug consumed, and drug remaining. This reconciliation was logged in the CRF ("Drug Accountability Log"). At the completion of the study, there was a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation was logged on the drug reconciliation form ("Drug Accountability Log"), signed and dated. Any discrepancies noted were investigated, resolved, and documented prior to return or destruction of unused study drug. Drugs destroyed on site were documented in the study files. The medication was to be used exclusively in the clinical trial according to the instructions of this trial protocol. The principal investigator confirmed the receipt of the trial medication with his signature. One copy of the receipt was kept at the site. Trial medication was stored in securely locked areas not generally accessible until dispensed to the patients. The medication was stored at insert in a temperature controlled, specifically assigned locker. All remaining trial medication was left in the original packaging and returned to the pharmacy at the end of the trial.

6.1.4 Treatment assignment

Patients were assigned to treatment only if they satisfied all the inclusion criteria and were not precluded from participation by any of the exclusion criteria.

Each patient, after the wash-out/run-in phase, was assigned to the open study medication according to the study protocol.

6.2 Therapy after completing the trial

After completing the trial the participants were advised to refer to their family physician for the continuous care of their diseases. This was explained to the study participants and the family physicians were informed by a letter written by the investigator at the last study visit of each study participant.

6.3 Concomitant therapy

All concomitant therapies were recorded throughout the study visits and kept constant, if possible. In particular stable therapy with respect to statins, antihypertensive medications, and antiplatelet agents was allowed.

RAAS blocking drugs were allowed at Visit 1 (screening) and then washed out during the next 4 weeks. After that, no additional RAAS blocking medication was allowed during the entire study.

6.4 Treatment compliance

Records of study medication used, dosages administered, and intervals between visits were kept during the study. Drug accountability was noted by the investigators.

7 Protocol amendments, other changes in study conduct

There was one substantial amendment in the study conduct: Additional measurement of renal hemodynamic after intravenous application of glucose; once before and once after medication with aliskiren.
This was notified to BfArM and Ethics Committee of the University of Erlangen, which approved it on 02-11-2012.

8 Statistical analysis

In this section, only the most important parts of the statistical analyses planned for this study are explained.

In general:

Protocol Deviations and their Classification in Minor and Major

Prior to locking the trial data base, possible protocol deviations were listed by a systematic data review. The classification into minor or major deviations was done in cooperation between the Sponsor's Project Manager and the responsible monitoring person of the subcontracted CRO. It was pre-specified that major protocol violations are protocol deviations which are considered to interfere with the assessments of efficacy in this trial.

Note: One protocol deviation was classified as a major protocol violation. For patient 121 from site Erlangen, PAH values at baseline clamp were not valid, so patient was excluded from PP population.

Note: One other protocol deviation was elevated liver parameters (ALAT > 100) of patient 18 from site Nürnberg throughout the study. This case was classified as clinical insignificant by the medical expert.

Missing Values

Missing values on efficacy data were not replaced.

Missing safety and tolerability data also were not replaced.

Data of patients having withdrawn their consent to study participation at any time point during the study were not accounted for in the respective analyses up to the time point of withdrawal.

Treatment Exposure and Compliance

Compliance by treatment and by visit

Records of study medication dispensed and returned and intervals between visits (in days) were kept during the study. Drug accountability was noted by the investigators.

The number of missing treatments was recorded per visit and the compliance was calculated directly by the investigator in the CRF as follows:

Compliance (%) = No. caps actually taken / No. caps to be taken * 100

This compliance by visit was described per treatment group and overall.

For all patients, compliance was found to lie in the proscribed range from 80 – 120%.

Exposure

Exposure time (in days) was calculated as follows:

Exposure (days) = date of the last treatment intake - date of first treatment intake + 1

8.1 Sample size and power considerations

The sample size determination was calculated by the Institute for Medical Statistics and Epidemiology of the Technical University Munich (Professor Dr. Ulm). Based on literature a 10 – 15% decrease of the GFR and the RPF was estimated. Thus 30 patients had to be evaluable, which meant 50 patients to be included for the needed number of per protocol evaluable patients (statistical considerations: α=0.05, β=0.80, SD = 9 %, effect size 6%).

8.2 Methods

All variables recorded in the CRF and all derived parameters were used in the statistical analysis.

For the analysis of our objectives derived in an open trial the recommendation was to focus on an unadjusted analysis, e.g. a simple paired t-test under the assumption of a normal distribution for the difference of interest in this case, as long as carry over effects can be neglected. By applying an analysis of covariates (ANCOVA) methods for repeated measurements, covariates that can vary over the time course of the study (e.g. change in body weight) can be integrated in the model. This approach takes care of potential confounding effects otherwise neglected.

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8.3 Analysis Sets

For the analyses of the study following populations were pre-specified.

Analysis Populations

The **Screened population (SCR population)** included all patients who provided informed consent and any demographic or baseline assessment (n=47).

15 patients were screened but never received any study drug. These will be excluded from the safety population.

The **Safety population (SAF population)** included all patients who recieved at least one dose of study medication. (n=32).

The **Intention-to-treat population (ITT population)**
The ITT Population included all patients who were randomised and received at least one dose of study medication and with any post-baseline efficacy evaluation (n= 32)

The **Per Protocol population (PP population)** included all patients of the ITT population who did not show any major protocol violation (n=31).

8.4 Demographic data and other baseline characteristics

Relevant data for the important baseline characteristics are summarized using suitable descriptive statistical procedures to describe the study population.

8.5 Safety

All SAE and AE were taken into account for safety analyses. The SAF population set was used for safety analysis.

8.6 Analysis Program

All statistical analyses were performed using Statistical Analysis System SPSS (release 19.0 SPSS Inc. Chicago, Illinois, USA).

8.6.1 Data management

The data in the project-specific database were exported to SAS records for further validation and analysis. In close collaboration with the responsible data manager and study statistician, CRC undertook data validation and plausibility checks as described in the data validation plan. Invalid, contradictory and/or improbable data were rechecked by the responsible personnel at CRC based on the CRF and corrected if possible.

8.6.2 Data check / Monitoring

The data were reviewed by CRC for completeness upon handover of the documentation. In addition, a further check for completeness was done after completion of the data entry by CRC. Particular attention was paid to the completeness of the data on AE.

8.6.3 Quality assurance measures

Safety laboratory markers were done by the accredited central lab of the University Hospital Erlangen.
Laboratory evaluations for PAH and IN were carried out by the laboratory of the Department of Nephrology and Hypertension, University of Erlangen-Nürnberg according to their SOP.
In addition, all study nurses were trained and experienced for at least 2 years and measurements were done under the supervision of Prof. Dr. Roland Schmieder.

9 Results

Please be aware: In this section, only results of the PP population are shown for reasons of clarity.

9.1 Time frame

The first patient to enter the study was enrolled on March 3rd, 2012. The final examination of the last patient was done on June 27th, 2013. Data base lock was end of December 2013.

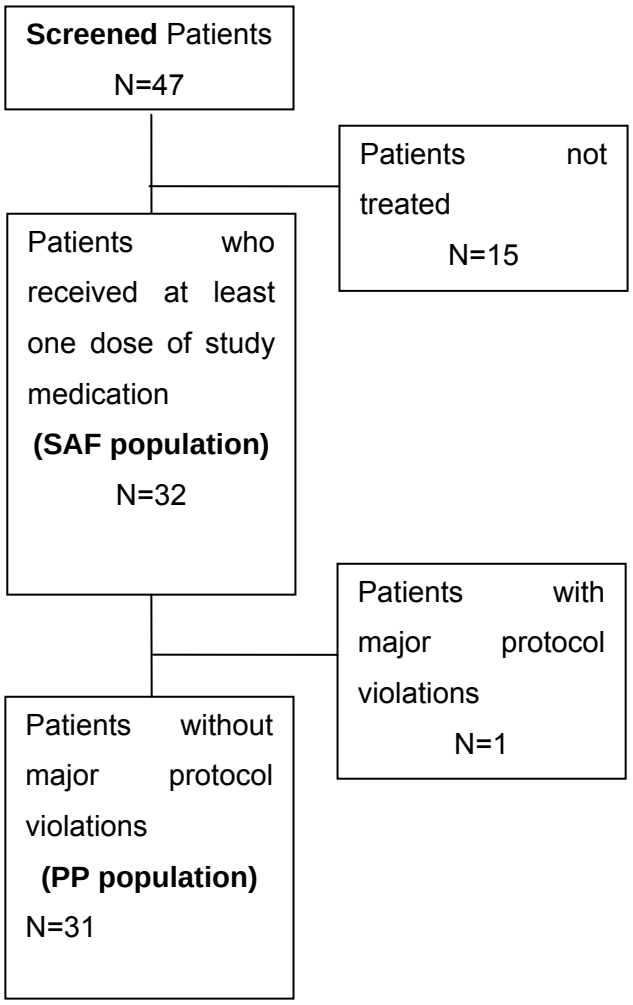
9.2 Baseline data

9.2.1 Patient disposition

The total number of screened patient was 47. Fifteen patients were screened but never received any study drug. These were excluded from the SAF population. The total number of aliskiren-treated patient was 32. One patient was excluded from the PP analysis due to invalid PAH values at baseline clamp.

Figure 9-1 Patient disposition

The following flow-chart summaries the study population “pre diabetics” considered for efficacy:



9.2.2 Demographics

The mean age of patients was 60.5 ± 7.7 years, 3 patients were female (9.7%). The average body mass index was 30.2 ± 3.3 kg/m^2. HbA1c prior to randomization was 5.89 % and mean blood pressure 148 / 88 mmHg.

9.3 Efficacy

9.3.1 Clinical characteristics

Table 9-1 describes the clinical characteristics of study patients before and after 5 weeks of aliskiren therapy (PP population, n =31).

Table 9-1 Clinical characteristics before and after 5 weeks of aliskiren therapy (PP population, n=31)

	Before aliskiren	After aliskiren	p-value
Weight (kg)	94.5 ± 14	94.1 ± 14	0.128
Office blood pressure (mmHg)			
Systolic	148 ± 11	134 ± 12	<0.001
Diastolic	88 ± 9	81 ± 8	<0.001
HbA1c (%)	5.89 ± 0.2	5.87 ± 0.2	0.753
Glucose fasting (mg/dl)	102 ± 8.2	102 ± 9.5	0.589
Creatinine (mg/dl)	0.85 ± 0.1	0.87 ± 0.1	0.294
High sensitive CRP (mg/L)	2.57 ± 2.7	2.43 ± 1.8	0.807
Lipids			
Total cholesterol (mg/dl)	222 ± 46	225 ± 41	0.685
LDL cholesterol (mg/dl)	153 ± 37	156 ± 35	0.495
HDL cholesterol (mg/dl)	49 ± 8.1	48 ± 8.5	0.524
Triglycerides (mg/dl)	145 ± 65	158 ± 88	0.228
Adiponectin (µg/ml)	5.40 ± 3.0	5.33 ± 2.9	0.658
HOMA Index	2.64 ± 1.3	2.79 ± 1.6	0.436
Insulin (mU/L)			
before glucose clamp	6.70 ± 2.7	7.49 ± 3.9	0.073
after glucose clamp	28.6 ± 18	31.3 ± 22	0.308

Legend: HbA1c, glycosylated haemoglobin, CRP, C reactive protein, LDL, low density lipoproteine, HDL, high density lipoprotein, HOMA,Homeostasis Model Assessment

Systolic and diastolic blood pressure was significantly reduced between before and after 5 weeks of aliskiren from hypertensive to normotensives values. All other parameters including lipid profile and insulin levels before and after glucose clamp didn´t change relevantly during the study conduct.

9.3.2 GFR and RPF by IN - /PAH - Clearance

Following the treatment with aliskiren absolute GFR values did not change, neither at baseline, nor during clamp. Glucose CLAMP lead to a 20 % increase of the GFR ($p < 0.001$), with no significant difference before and after 5 weeks aliskiren therapy.

A significant increase of RPF was shown in prediabetic subjects after 5 weeks of treatment with aliskiren, at baseline and during hyperglycemic clamp-test (p=0.020). The change due the hyperclycemic clamp test was not altered (p=0.722).

Table 9-2 Renal hemodynamics before and after 5 weeks of aliskiren therapy (PP population, n=31)

	Before aliskiren	**After aliskiren**	**P -Value**
GFR, ml/min*			
Baseline	101 ± 14	101 ± 13	0.847
After CLAMP	121 ± 15	123 ± 16	0.313
Delta GFR (after – before CLAMP)	19.8 ± 7.9	22.1 ± 6.1	0.188
Delta GFR, %	20.0 ± 7.7	22.2 ± 6.3	0.233
RPF, ml/min*			
Baseline	412 ± 72	452 ± 101	0.001
After CLAMP	494 ± 96	531± 114	0.020
Delta RPF (after – before CLAMP)	82.1 ± 63	78.9 ± 38	0.722
Delta RPF, %	20.5 ± 17	18.0 ± 8.0	0.435
FF, %*			
Baseline	20.1 ± 3.9	18.3 ± 2.2	0.003
After CLAMP	20.1 ± 3.2	19.0 ± 2.3	0.313
Delta FF (after – before CLAMP)	-0.04 ± 2.4	0.66 ± 1.2	0.150

Delta FF, %	0.65 ± 9.9	3.85 ± 6.6	0.122

*Legend: GFR, glomerular filtration rate, RPF, renal plasma flow, FF, filtration fraction, * adjusted to BSA (Body surface area)*

FF decreased after 5 weeks of treatment with aliskiren (p=0.003). The response of FF to the hyperglycemic clamp was not altered (p=0.313).

10 Safety

10.1 Adverse and serious adverse events

In total, 30 AEs were reported during the study conduct (see Table 10-1). No patients discontinued their study participation due to drug-related SADR as recorded on the termination form.

No SAE occurred during the study.

Table 10-1 Number of adverse and serious adverse events of SAF population (n=32).

	Aliskiren	
Adverse event (AE)	**n**	**%**
All AE	**30**	
Patients with AE	**17**	
Serious AE	**0**	
Treatment disc. due to AE	0	
Headache	11	37
Increased micturition	1	3
Heart Burn	1	3
Common cold	2	7
Increased allergy	1	3
Abdominal pain	1	3
Obstipation	1	3
Eyestrain	1	3

Thoracic pain	1	3
Pain left ankle	1	3
Macrohematuira	1	3
Diarrhea	3	10
Dizziness, mild	1	3
Intermittent paresthesia left thoracal	1	3
Cough	1	3
Flatulence	1	3
Redness left arm	1	3

10.2 Serious Adverse Drug Reactions

No serious suspected adverse drug reaction was recorded.

10.3 Changes in laboratory parameters during the study conduct

During the study blood samples were drawn at pre-specified visits (V1, V3, V4, V5, V6) to determine the parameters, listed in Table 10-2 as far as they are not already listed as primary or secondary endpoints or given in Table 9-1.
Safety laboratory values included liver, renal, haematological and urine parameters (not reported).

Please be aware: Also in this section, only results of the PP population are shown for reasons of clarity.

Table 10-2 Mean values in laboratory parameters before and after 5 weeks of aliskiren therapy (PP population, n=31)

Visit	**Before Aliskiren**	**After Aliskiren**	**P – Value**
Liver parameters			
SGOT (U/L)	26.3 ± 9.5	27.1 ± 10	0.482
SGPT (U/L)	33.5 ± 19	34.7 ± 19	0.605
Gamma-GT (U/L)	40.3 ± 30	40.9 ± 24	0.754

AP (U/L)	66.4 ± 17	65.9 ± 14	0.725
Bilirubin (mg/dl)	0.73 ± 0.2	0.65 ± 0.2	0.037*
Elektrolytes			
Sodium (mmol/L)	139 ± 1.6	138 ± 1.8	0.233
Potassium (mmol/L)	3.98 ± 0.2	4.07± 0.2	0.080
Calcium (mmol/L)	2.31 ± 0.1	2.31 ± 0.1	0.835
Other lab			
Urea (mg/dl)	32.8 ± 6.3	33.8 ± 7.1	0.376
Uric acid (mg/dl)	6.26 ± 1.3	6.29 ± 1.1	0.850
Hematological parameters			
WBC (/µl)	6.06 ± 1.3	5.68 ± 1.0	0.027*
Hb (g/dl)	14.3 ± 1.1	14.4 ± 0.9	0.739
Platelets (/µl)	211 ± 46	210 ± 45	0.865
RBC (/µl)	4.57 ± 0.4	4.55 ± 0.3	0.515
Hematocrit (%)	42.2 ± 3.1	42.1 ± 2.8	0.880

*Legend: *judged as medical*

not relevant, SGOT, glutamat-oxalacetat-transaminase, SGPT, glutamat-Pyruvat-transaminase GT, Glutamyltransferase, AP, alkalic phosphatase, WBC, white blood cell count; Hb, haemoglobin; RBC, red blood cell count; Hkt, haematocrit

11 Discussion and Summary

The key results of the present study are:

- A significant increase of RPF was shown in prediabetic subjects after 5 weeks of treatment with aliskiren before and during hyperglycemic clamp test.
- GFR showed no changes under aliskiren treatment neither at baseline nor during hyperglycemic clamp test.
- The changes (Delta RPF and Delta GFR) between baseline and during hyperglycemic clamp test did not show any differences in RPF as well as in GFR.
- No changes in metabolic and inflammatory parameters were recorded during treatment with aliskiren.

- Treatment with aliskiren 150 mg/d for one week and 300 mg/d for four more weeks in pre-diabetic patients was safe and well tolerated.

The study was to our knowledge the first to have analyzed renal hemodynamics by PAH/IN-clearance during an acute glucose stress-test in pre-diabetic patients before and after therapy with aliskiren. At baseline we observed increased renal perfusion but no glomerular hyperfiltration in prediabetes under aliskiren treatment.

Our data strengthen earlier findings on renal hyperperfusion during aliskiren therapy [7], which were employed by measuring renal perfusion by 1.5 T magnetic resonance imaging-arterial spin labeling in subjects with arterial hypertension before and after therapy with aliskiren (4 weeks of treatment with 300mg). Aliskiren significantly increased renal perfusion (p=0.03).

An increase of RPF has also been observed in 10 insulin-resistant and 10 insulin-sensitive normal subjects during physiological and supraphysiological hyperinsulinemia. Patients underwent the technique of I-labelled hippuran clearance. In insulin-sensitive patients RPF increased more than in insulin-resistant ones. Under supraphysiological conditions both groups increased to comparable levels [8]. The same effects were shown in experimental studies, i.e. the increase of renal blood flow (RBF) by insulin infusion in sheep [9]. Both studies discussed similar reasons for the increase of RPF and RBF, which are especially ascribed to the effect of vasodilatation of insulin, which is nitric oxide (NO) mediated.
These studies therefore point to the pathogenetic mechanism that the increase in renal perfusion after aliskiren might be related to improved NO availability by blocking the RAS. This effect has been described for angiotensin receptor blockers. The vasodilatory effect of insulin secretion provoked by the hyperglycemic clamp test seems not being further altered after aliskiren in prediabetic patients.
Further research is underway to put the finding of renal hyperperfusion in prediabetic subjects into perspective and comparison to healthy and insulin resistant patients as a possibly specific attribute of this patient group.

12 Conclusion

Aliskiren treatment increases RPF at baseline and during hyperglycemic clamp-test in pre-diabetic patients, whereas GFR showed no differences before and after aliskiren.

The changes (Delta RPF and Delta GFR) between baseline and clamp emerged no differences in RPF as well as in GFR. A 5 week treatment with aliskiren is safe and does not change metabolic and inflammatory parameters.

13 References

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14 Appendixes

14.1 Clinical Trial Protocol

14.2 CRF