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KLINIK FÜR DERMATOLOGIE,
VENEROLOGIE UND ALLERGOLOGIE
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Klinik mit zertifiziertem Qualitätsmanagementsystem nach DIN EN ISO 9001:2000

ALLERGIE-CENTRUM-CHARITÉ
Bereich Klinische Studien

Prof. Dr. med. Margitta Worm

Explorative analysis of the immunmodulatory capacities of apathogenic Escherichia coli Nissle 1917 in patients with rhinoconjunctivitis due to grass pollen allergy.

In German:
Explorative Untersuchung des immunmodulatorischen Effektes von apathogenen *Escherichia* coli Nissle 1917 bei Patienten mit allergischer Rhinokonjunktivitis durch Gräserpollen

Investigational product:	**E. coli Nissle 1917 (Mutaflor®)**
Eudra-CT number:	**2008-006335-12**
Protocol-code:	**Ecorhino**
Indication studied:	**seasonal allergic rhinoconjunctivitis to grass pollen**
Development phase:	**therapeutic exploratory (Phase II)**
Trial dates:	**start: 3rd February 2009** **end: 6th October 2009**

Clinical Trial Report

Version 1.0 / date: 06.10.2010

Sponsor/Principal Investigator of the clinical trial/Author

_____Prof. Dr. med. Margitta Worm_____

Project Coordination/Author:

_____Dipl. troph. Sabine Dölle_____

Signature Page

- Confidentiality -

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All the following persons have read this clinical trial report and confirm that to the best of their knowledge it accurately describes the conduct and results of the clinical trial.

Sponsor/Principal Investigator/Author

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Project coordinator/Author

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2 Synopsis

Title	Explorative analysis of the immunmodulatory capacities of apathogenic Escherichia coli Nissle 1917 in patients with rhino-conjunctivitis due to grass pollen allergy.
Development phase of the study	exploratory trial (therapeutic exploratory phase II)
Sponsor / Principal Investigator	Prof. Dr. med. Margitta Worm Charité – Universitätsmedizin Berlin Department of Dermatology and Allergology Allergy-Center-Charité (ACC) Charitéplatz 1, 10117 Berlin, Germany Phone: +49 (0)30-450 518 105 Fax: +49 (0)30-450 518 931 margitta.worm@charite.de
Study centres:	mono-centre
Publication (reference)	manuscript in preparation
Study period **Date of first patient enrolled:** **Date of last patient completed:**	 3rd February 2009 6th October 2009
Study objectives	The clinical trial aimed to assess the immunmodulatory effect of EcN on the immune system and on the clinical symptoms of patients with seasonal AR to grass pollen.
Primary objectives	Symptom-medication-score (SMS), determined over one grass pollen season, in an exploratory manner compared to placebo.
Secondary objectives	- medication score alone - symptom score alone - conjunctival provocation test (CPT) - skin prick test (SPT) - rhinoconjunctivitis quality of life questionnaire (RQLQ) - number of symptomfree days - global evaluation of rhinoconjunctivitis symptoms
Study design, time table, …	prospective, randomised, double-blind, placebo-controlled, two-armed, parallel-designed
Investigational product	E. coli Stamm Nissle 1917 (EcN), trade name Mutaflor® 1 capsule contains 2.5 – 25 billion viable bacteria of the strain E. coli Nissle 1917 oral application
Comparator	Placebo, apart from the active substance (EcN) identical
Intervention	6 months oral supplementation
Study population	Planned: 34 Included: 34 Drop-outs: 4 Analyzed: 30 (16 EcN-group; 14 placebo-group)

Main inclusion criteria	• Age between 18 and 65 years (women and men) • Clinical relevant grass pollen allergy with required treatment of the rhinoconjunctivitis symptoms since at least 2 years • Positive skin prick test (SPT) to grass pollen extract • Positive specific IgE towards grass pollen (at least CAP class 2) • Retrospective symptom-score (rSS) (at least 12 points) • Reliable method of contraception for women of childbearing potential • Written informed consent according to AMG §40 (1) 3b
Efficacy conclusion	The primary efficacy parameter, the SMS failed to be statistically significant different between placebo and EcN group. Thus, the hypothesis -EcN has immunmodulatory capacities- could not be confirmed in the investigated patient's collective. The secondary efficacy parameter did not show any significant differences between placebo and EcN. However, some parameters were different among the groups (CPT, global evaluation).
Safety conclusion	The blinded EcN supplementation was safe and well tolerated. The EcN-related AEs were all located in gastrointestinal tract and mainly mild in its intensity. It is known that these symptoms (flatulence, abdominal pain, diarrhoea) might occur in the beginning of Mutaflor® intake, but resolve by when the patient got used to it.

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3 List of Abbreviations and Definitions of Terms

AE	adverse event
AMG	Arzneimittelgesetz (German drug law)
AR	allergic rhinoconjunctivitis
AUC	area under the curve
ALT	alanine aminotransferase
AP	alkaline phosphatase
CPR	conjunctival provocation test
CRF	case report form
EcN	*Escherichia coli* Nissle 1917
GCP	good clinical practice
GGT	gamma glutamyltransferase
ICH	international conference of harmonisation
IEC	independent ethics committee
IMP	investigational medicinal product
ITT	intention-to-treat
PP	per-protocol
SAE	severe adverse event
SD	source date
SmPC	summary of product characteristics
SMS	symptom medication score
SPT	skin prick test
SOP	standard operating procedures
RQLQ	rhinoconjunctivitis quality of life questionnaire
rSS	retrospective symptom score
TMF	trial master file

4 Ethics

4.1 Independent Ethics Committee (IEC)

The protocol of the clinical trial, the patient information and informed consent, and any other written information provided to the patients was approved by the local Independent Ethics Committee (IEC) of Berlin.

The principal investigator (here also sponsor: Prof. Dr. med. M. Worm) was responsible for submitting the documents to the IEC of Berlin.

During the trial no documents were sent to the IEC for reviewing (no amendment).

At the end of the clinical trial, the sponsor/principal investigator notified the IEC of Berlin about the trial completion. The synopsis of the final report will be provided to the IEC within approximately 30 days after signing of the final report.

The address and chairmen of the IECs are given in section 5.

4.2 Ethical conduct of the clinical trial

The clinical trial was conducted in accordance with applicable regulations governing the protection of human patients, such as national drug laws (German Drug Law: AMG [1]), ICH-GCP guidelines [2; 3] and the Declaration of Helsinki [4].

According to the AMG, the sponsor/principal investigator covered insurance for all patients who gave informed consent to this clinical trial.

4.3 Patient information and informed consent

IEC approval of the written patient information and informed consent was obtained prior to their use. The informed consent contains a phrase by which consent was given for the access to the non-personalized data by the sponsor, national and regulatory authorities. In addition, it states that the patient was free to withdraw from the clinical trial at any time without any negative consequences. The patient information gives a complete and comprehensive explanation of the significance, nature, extent and possible risks of the clinical trial. Additionally to the written patient information, oral informing was done by the investigator. It complied with all applicable regulations governing the protection of human patients, such as national drug laws (German Drug Law: AMG [1], ICH-GCP guidelines [2; 3] and the Declaration of Helsinki [4].

A sample of patient information and informed consent are provided in appendix 15.1.1.

5 Investigators and Trial Administrative Structure

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Leading Ethics Committee
Landesamt für Gesundheit und Soziales Berlin (LaGeSo)
Agency of the ethic committee of Berlin
Chairman: PD Dr. med. Martin Hildebrandt (commission 5)
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For the chairmen and member of the responsible committee of the local IEC participating in the clinical trial, please refer to appendix 15.1.3.

The curricula vitae of the investigators and other important participants of the clinical trial are provided in appendix 15.1.4.

6 Introduction

Due to the worldwide increasing prevalence of allergic rhinoconjunctivitis (AR), new therapeutical strategies are needed. The symptomatic treatment with topical and systemic antihistamines and corticosteroids are often insufficient. *Escherichia coli* Nissle 1917 (EcN) has immunmodulatory capacities and is well tolerated. EcN has no sedative properties and exhibits no hepatotoxic and nephrotoxic qualities. Thus, EcN represents a new relevant therapeutical agent.
The immunmodulatory effect of EcN on the immune system and the clinical symptoms of patients with seasonal AR to grass pollen were assessed in an exploratory clinical trial in comparison to placebo. The primary endpoint was the symptom-medication score (SMS) in the grass pollen season.

The clinical trial protocol was conducted to perform the clinical trial according to the legal requirements, and to the current revisions of the recommendations of the Declaration of Helsinki [4], Good clinical Practice (GCP)- and International Conference on Harmonization (ICH)-guidelines [2; 3].

7 Study objectives

The clinical trial aimed to assess the immunmodulatory effect of EcN on the immune system and on the clinical symptoms of patients with seasonal AR to grass pollen.

8 Investigational plan

8.1 Overall study design and plan: description

Patients with diagnosed AR with necessarily medical treatment since at least two years were recruited. The clinical trial was conducted as a prospective, randomised, double-blind, placebo-controlled, two-armed, parallel-designed, exploratory trial (therapeutic exploratory phase II). The two arms represent the EcN-group or the placebo-group, respectively (Fig. 13-1)

Patients underwent complete medical history, physical examination, and laboratory evaluation before treatment started. Eligibility was checked by the investigator on the basis of the in- and exclusion criteria.

The AR were diagnosed by case history, positive skin prick test (SPT) and specific IgE level against grass pollen of at least CAP 2 (>0.70 kU/l).

A sample size of 34 patients was calculated in advance (chapter 8.7.2). During the screening phase, 46 patients had been assessed for eligibility to reach 34 patients. The randomisation was done by the biometrician of the KKS Charité (chapter 8.4.3). In each group, 17 patients

were enrolled and constituted the intention-to-treat (ITT) population. Finally, 30 patients were analysed (16 in the EcN- and 14 in the placebo-group). The full study flow chart is depicted in Fig. 13-2.

The screening phase was performed in February 2009. The EcN supplementation started within the first week of March 2009. The first 8 weeks were defined as a wash-in phase and with the start of the grass pollen season the EcN supplementation was continued for 4 more months. In total, the EcN supplementation was carried out over 6 months. A follow-up was performed in October 2009. The clinical trial was finished with the last follow-up of the last patient, 6th October 2009 (Fig. 13-3).

Trial medication was administrated orally, 1 capsule per day for the first 4 days and afterwards 2 capsules per day until the end of supplementation. One capsule contains 2.5 - 25 billion viable bacteria of the strain *E. coli* Nissle 1917.

8.2 Discussion of study design, including the choice of control group

The clinical trial was designed to evaluate the immunmodulatory action of EcN in grass pollen allergic patients. Due to the worldwide increasing prevalence of AR, new therapeutical strategies are needed. The symptomatic treatment with topical and systemic antihistamines and corticosteroids are often insufficient. EcN has immunmodulatory capacities and is well tolerated. EcN has no sedative properties and exhibits no hepatotoxic and nephrotoxic qualities. Thus, EcN represents a new relevant therapeutical agent.

This clinical trial was conduced placebo-controlled. The patients had controlled access to the standard medication comprising antihistamines, nasal steroids, inhalant β_2-adrenergic receptor agonist and steroids. Thus, no patient was kept to use the standard symptomatic therapy.

The wash-in phase of 8 weeks was chosen according to previous studies from the literature [5, 6].

The dosage of EcN was used equivalent to the use of Mutaflor® for long-term treatment as described in the summary of product characteristics (SmPC). The overall duration of supplementation was chosen according to the expected grass pollen season and did not exceed the maximal duration according to the SmPC.

The exploratory character was selected as this is the first administration of EcN in patients with AR due to grass pollen. The application of EcN in this disease was examined in a limited sample size to estimate the intensity of immunmodulatory effect of EcN in patients with AR.

8.3 Selection of study population

8.3.1 Inclusion criteria

- Age between 18 and 65 years (women and men)
- Clinical relevant grass pollen allergy with required treatment of the rhinoconjunctivitis symptoms since at least 2 years
- Positive skin prick test (SPT) to grass pollen extract
- Positive specific IgE towards grass pollen (at least CAP class 2)
- Retrospective symptom-score (rSS) (at least 12 points)
- Reliable method of contraception for women of childbearing potential
- Written informed consent according to AMG §40 (1) 3b

8.3.2 Exclusion criteria

Patients meeting any of the following exclusion criteria were not to be included into the trial.
General exclusion criteria:

- Pregnancy or lactation
- Participation in another clinical trial within the last 30 days

- Subjects who are inmates of psychiatric wards, prisons, or other state institutions (according AMG §40 (1) 4)
- Clinically significant laboratory abnormalities (biochemistry and haematology)
- Other reasons like mental disorders, Drug or alcohol dependency

Medical exclusion criteria:
- Perennial rhinoconjunctivitis
- Chronic diarrhoea and other existing severe gastrointestinal diseases
- Chronic sinusitis
- Bronchial asthma (Gina II - IV)
- Severe cardiovascular diseases and metabolic disorders, autoimmune diseases or other systemic inflammatory disorders
- Known hypersensitivity to trial medication/placebo or their components or to the rescue medication

Prohibited concomitant medication:
- Use of Mutaflor® **12 weeks** before the start of the trial
- Use of antibiotics or sulfonamides towards gram negative bacteria **4 weeks** before the start of the trial
- Specific immunotherapy to grass pollen **6 months** before the start of the trial
- Current specific immunotherapy to any allergen
- Immunosuppressive therapy

8.3.3 Removal of patients from treatment or assessment

The criteria for withdrawal of a subject from the clinical trial were the following:
- Personal desire of the patient
- Pregnancy
- Non-compliance
- Severe diarrhoea
- Use of antibiotics or sulfonamides towards gram negative bacteria
- Long-lasting severe adverse reactions like nausea, vomiting, dermal adverse reactions
- Any other situation which might make the further participation of the patient difficult or unethical (investigator's decision)
- Unblinding in the case of medical emergency

In the clinical trial Ecorhino four patients dropped out:
- one because of adverse event (p-no. 30)
- one because of protocol deviation (steroid injection, p-no. 3)
- one because of not showing up again (moved from Berlin to Hamburg, p-no. 11)
- one because of not showing up again (patient did not appear to visit 4, the study team tried several times to approach the patient via e-mail and telephone unsuccessfully, p-no. 31)

Lost by follow-up were no patients.

Four patients were excluded from the efficacy assessment:
- three patients because of drop out (p-no. 3, 30, 31)
- one because of a planned operation of the nose and use of postoperative nasal steroids

The one patient (p-no. 11) who dropped out was included in the efficacy assessment because the data set of the primary efficacy parameter (SMS) was complete.

8.4 Treatments

8.4.1 Treatments administration

The investigational medicinal product (IMP), Mutaflor® is a product marketed by Ardeypharm GmbH and contains the active ingredient viable bacteria of the strain *E. coli* Nissle 1917 (approval number: 6091994.00.00).

As comparator a placebo was chosen which was completely identical to the IMP without containing EcN.

The IMP and placebo were provided and blinded by Ardeypharm GmbH. The randomisation was done by the KKS Charité.

8.4.2 Identity of the investigational product

The trial medication (IMP, placebo) was given orally in a dosage of 1 capsule once daily in the first 4 days and 2 capsules per day from day 5. The whole supplementation period was 6 months. All patients received an introduction how to use the trial medication and had to document the daily intake in their patient's diary besides the documentation of tolerability. One group of patient received EcN and the other group placebo.

IMP

Product name:	*E. coli* Stamm Nissle 1917 (EcN), trade name Mutaflor ®
Manufacturer*:*	Ardeypharm GmbH, Herdecke
Active substance:	one capsule contains 2.5 – 25 billion viable bacteria of the strain *E. coli* Nissle 1917
Other ingredients:	maltodextrin, talcum, Poly(methacryle acid-co-methylacrylate), Macrogol (4000), triethyl citrate, glycerin 85%, titanium dioxide, iron(III)-hydroxide-oxide x H_2O, gelatine, yellow wax, carnauba wax,, shellac, purified water

Placebo

Manufacturer*:*	Ardeypharm GmbH, Herdecke
Active substance:	none
Other ingredients:	maltodextrin, talcum, Poly(methacryle acid-co-methylacrylate), Macrogol (4000), triethyl citrate, glycerin 85%, titanium dioxide, iron(III)-hydroxide-oxide x H_2O, gelatine, yellow wax, carnauba wax,, shellac, purified water

The IMP and placebo were apart from the active substance identical and similar in the outward appearance.

The trial medication was packed in tubes filled with either 60 or 80 capsules and all tubes had the same batch number: 872010.

The expiry date was different:

	Expiry date
Patient 1-36 tube 1 and 2	27th July 2009
Patient 1-29 and 31-34 tube 3 and 4	24th August 2009
Patient 1-2, 4-29 and 31-34 tube 5 and 6	5th November 2009

The trial medication had to be stored at +2 to +8℃ . This was controlled by a temperature log over the whole study period. The patients were instructed to store the trial medication in their fridge and were equipped with cool packs for transporting.

For detailed information about the labelling of trial medication see appendix 15.1.5.

8.4.3 Method of assigning patients to study medication

All patients were recruited in the Allergy-Centre-Charité as this was a mono-centre trial. Announcements were done over the bulletin board of the Charité and a regional daily newspaper (Berliner Zeitung). Recruited patients obtained a screening and if applicable a patient number (=random number) in ascending sequence.

A randomization list assigned the different treatments (EcN or placebo) and is provided in appendix 15.1.6 including the patient identifier, and treatment assigned. The randomization list was created by the KKS using the software "RandList 1.2" according to the manufacturer's instruction (company: DatInf). A block randomization was performed with a block size of 4. Thus, 36 random numbers were created. No stratification was included. After patients enrolment the random number was assigned in ascending sequence to the patients.

8.4.4 Selection of dose in the clinical trial

Since, there is limited data on the immunmodulatory effect of EcN in AR, we used the previously approved dosage of Mutaflor® (approved indication: colitis ulcerative in remission and chronic obstipation). Thus, the patients were introduced to take 1 capsule per day for the first 4 days and 2 capsules once daily from day 5. The overall supplementation period of 6 months was within the approved therapeutical use of Mutaflor®.
The patients were introduced to consume the capsules once daily if possible during breakfast.

8.4.5 Blinding

The IMP and placebo were identical apart from the active substance, the billion viable bacteria of the strain E. coli Nissle 1917. Both substances were provided in the pharmaceutical form of gastro-resistant hard capsules packed in tubes of 60 or 80 capsules.
The blinding, packing and labelling were done by Ardypharm GmbH.

Two sets of sealed envelopes were prepared by Ardeypharm GmbH according to the randomization list which was provided to Ardeypharm by the biometrician of the KKS Charité. One set was send to the study centre of Prof. Worm and was stored in the ISF. The randomization list was kept by Ardeypharm GmbH until the database was closed.

In this clinical trial no premature unblinding was necessary.

8.4.6 Prior and concomitant therapy

For prohibited concomitant medication see chapter 8.3.2.
Any other concomitant medications taken during the clinical trial or any changes in concomitant medication were documented in the CRF page concomitant medication indicating the:

- trade name of medication
- indication for use
- route of administration
- daily dose
- start date
- end date
- was the reason an AE
- on-going

A list of concomitant medication per patient can be found in appendix table 15.2.14 and the CRF page of concomitant medication in appendix 15.1.2.

8.4.7 Treatment compliance

The treatment compliance was controlled by diary cards, which had to be filled in every day by the patient and were inspected at every visit by the investigator. In the diary cards, the

patients had to document any changes in their health or concomitant medications and had to assess the tolerability of the trial medication. An example of diary card is provided in appendix 15.1.2. The compliance was additionally monitored by checking the consumed capsules when the tubes were returned.

8.5 Efficacy and Safety Variables

8.5.1 Primary Efficacy Variable

As primary endpoint the symptom-medication-score (SMS) was chosen to show the immunmodulatory effect of EcN on the clinical symptoms. The SMS was determined over one grass pollen season in an exploratory manner compared to placebo. The SMS is calculated from 10 different allergic symptoms (including nose, eye and lung symptoms) and the use of allergic medication (antihistamines, cromoglycine eye drops, steroidal nasal spray, beta-2-mimetics, steroids). Details about the assessment of symptoms and medication are given in the protocol chapter 7.6.1.
The SMS was assessed daily by the patients and documented in the diary. The documentation period covered 6 months. The exact period of analysis was defined as 14 days before and 31 days after the grass pollen peak. Thus, the area under the curve (AUC) of the daily SMS over 45 days were calculated for the EcN and Placebo-group and were compared.
The grass pollen count for the year 2009 was obtained from the foundation of German pollen information service (Stiftung Deutscher Polleninformationdienst). The overall grass pollen count for Berlin, Germany 2009 is depicted in Fig. 13-4.

8.5.2 Secondary Efficacy Variables

The immunmodulatory effect of EcN was additionally assessed by the following variables:
- medication score alone
- symptom score alone
- conjunctival provocation test (CPT)
- skin prick test (SPT)
- rhinoconjunctivitis quality of life questionnaire (RQLQ)
- number of symptomfree[1] days
- global evaluation of rhinoconjunctivitis symptoms

8.5.3 Variables for safety and tolerability

Safety was assessed by the following:
- case history (general and allergic)
- safety laboratory and pregnancy test
- patient's diary documentation, including tolerability evaluation by the patient
- physical examination
- lung function test
- recording of adverse events by the investigator

The tolerability was assessed on a 4-point scale (1 – very good, 2 – good, 3 – moderate, 4 – bad) after the treatment period (visit 5).

8.5.4 Flow Chart

A study flow chart with all visits and assessments are depicted in Tab. 13-1.

[1] A symptomfree day is defined as a day without the use of allergic medication and a symptom score ≤2 points.

8.5.5 Appropriateness of Measurements

According to the EMEA guideline ("Guideline on the clinical development of products for specific immunotherapy for treatment of allergic diseases."[2]) the SMS was chosen as the primary efficacy parameter.

8.6 Data quality assurance

Data quality control and assurance were performed according to international guidelines (GCP, ICH), standard operating procedures (SOP) or working instructions. The data were documented first in the source data (SD) and afterwards in the case report form (CRF) by the investigator or designed personnel. The data were entered in a database and checked by a trial independent person. Once all data were entered in the data base, a blind data review meeting was assembled. The database was locked and released for reporting and statistical evaluations after all data quality control steps defined in the blind data review meeting were performed.

8.6.1 Monitoring (Quality control)

The KKS Charité was delegated to perform the monitoring. Four monitoring visits were done according to a monitoring visit plan and four monitoring reports were prepared.

8.6.2 Audit (Quality assurance)

External audits were not performed on this clinical trial.

8.7 Statistical Methods Planned in the Protocol and Determination of Sample size

8.7.1 Statistical and Analytical Plans

All data obtained in this clinical trial and documented in the CRFs and patient's diaries were analyzed with descriptive group statistics. Details of statistical analyses are found in the statistical analysis plan (appendix 15.1.8)
All randomised subjects who started the blinded EcN supplementation with the trial medication represent the ITT population. Safety analysis was performed with the ITT population.
The primary and secondary efficacy analyses were performed with the per-protocol (PP) population. It was intended to exclude the drop-outs from the PP population. There were 2 exceptions (see chapter 9.1) defined in the blind data review meeting. One patient who dropped out (p-no. 11) was still included in the PP population because the complete data set for the SMS was present. Another patient (p-no. 1) was excluded from the PP population because of postoperative regular use of nasal steroids over a longer period during the 45-day analysis time (see chapter 8.5.1).

8.7.2 Sample Size

A formal sample size calculation was not performed for this exploratory clinical trial. Due to the exploratory character it was defined to include 30 patients (15 per group). No previous data about the anticipated immunmodulatory effect of EcN in allergic diseases was available.
Four scenarios were performed to get information on the statistical power. A drop-out rate of 10% was expected. Thus 34 patients had to be randomised.

[2] http://www.emea.europa.eu/inspections/GCPgeneral.html

8.8 Changes in the Conduct of the Study or Planned Analyses

The clinical trial was conducted according to the clinical trial protocol; version 1.1 dated 15th December 2008 (appendix 15.1.1). No further formal protocol amendments were made.

9 Study population

9.1 Disposition of patients

Forty-six patients with diagnosed AR were screened for trial participation. Twelve patients were excluded during the interview with the investigator (screening failures, appendix 15.2.1).

Overall, 34 patients were enrolled and supplemented with EcN or placebo. Thirty patients completed the clinical trial, 4 patients were withdrawn prematurely. An overview over the patients is provided in the CONSORT flow diagram (Fig. 13-2).

The first signed informed consent was on 3rd of February 2009 (patient: S01) and the first randomisation was on 13th of March 2009 (patient: S46). Last patient and last visit was on the 6th of October 2009 (patient: S33).
All reasons and dates of dropped out patients after randomisation and the excluded patient during the blind data review meeting are listened in the appendix 15.2.2 and 15.2.3.

9.2 Protocol deviations

Only one patient (p-no. 3) broke the protocol and was excluded from the clinical trial. The patient is listed in appendix table 15.2.2. One drop out was included in the efficacy analysis because of a completed data set for the primary efficacy parameter (SMS).

9.3 Data sets analysis

The ITT population contains all patients who were randomized and who received the trial medication (34 patients). The analysis of the safety data was performed with the ITT-Group.
The PP population contains only the patients who fulfilled all protocol criteria and was fixed during the blind data review meeting on the 24th of November 2009 (30 patients). One patient (p-no. 1) was excluded from the PP population because of postoperative regular nasal steroid use over a longer period during the 45-day analysis time. One drop out-patient (p-no.11) was included because the completed data set for the primary efficacy parameter (SMS) was available. All efficacy analyses were performed on the PP population.

9.4 Demographic and other baseline characteristics

The age of the 5 male and 12 female patients of the EcN group ranged from 20 to 54 years and in the placebo group (8 females, 9 males) from 19 to 49 years (Tab. 13-2). All patients were Caucasian.

Only patients with moderate or severe AR with at least 12 points in the rSS, with positive SPT (≥3 mm) and sIgE CAP class ≥2 were included. The median for the rSS, the SPT, and sIgE are listed in the baseline characteristics (Tab. 13-2).

The safety laboratory parameters of all patients were normal before starting the clinical intervention. Since it is recommended to monitor kidney and liver function parameters (see SmPC), the main safety laboratory parameters creatinine and liver enzymes (ALT, AP, GGT) are tabulated in the baseline characteristics (Tab. 13-2).

Individual listings of demographic information and adherence to inclusion and exclusion criteria are provided in appendix table 15.2.18.

10 Efficacy evaluation

10.1 Measurement of treatment compliance

Compliance was checked by record of the daily intake of the capsules in the patient diary and was regularly checked by the sub-investigator. The overall compliance was very good. In all treatment weeks all patients used the trial medication correct. No patient discontinued the treatment for more than 10 days. The patients were asked to document their daily capsule intake, their daily symptoms on a scale, the use of anti-allergic medication, other not yet reported medication and remarkable events like a disease or holidays.

Another measurement of treatment compliance was counting the capsules which were collected on each visit and comparing the documented number with the statements in the patient diaries.

10.2 Efficacy Results and Tabulations of Individual Patient Data

10.2.1 Analysis of efficacy

10.2.1.1 Primary efficacy parameter

As primary efficacy parameter the rhinoconjunctivitis symptom-medication-score (SMS) was measured for a pre-defined time period of 45 days during the grass pollen season 2009 (see chapter 8.5.1). Grass pollen peak with 60 pollens per m³ air was on the 2nd June 2009. The SMS in the placebo group was 243.39 ±53.98 (area under the curve, AUC) and 326.99 ±53.58 (AUC) in the EcN group (Fig. 10-1).

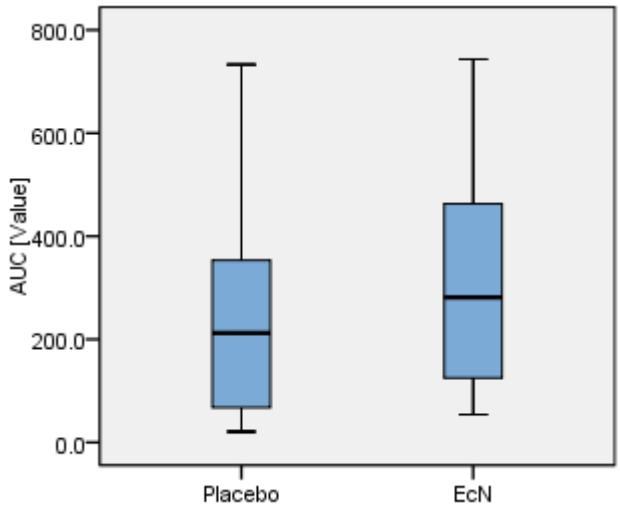

Fig. 10-1: SMS depicted as mean AUC.

In general, the grass pollen season 2009 was weak. The grass pollen peak was half as much as the grass pollen peak 2008 (see Fig. 13-5). Both treatment groups experienced rhinoconjunctivitis symptoms from the very beginning and took anti-allergic medications. Thus, both groups had a SMS >0 points (EcN [mean] = 1.44; Placebo [mean] = 2.43 points).

The EcN group had slightly higher SMS values (median: 5.0 points [0.0-22.0]) compared to the placebo group (mean: 3.5 points [0.0-18.0]) at the first day of the defined grass pollen season (20th May 2009). This circumstance did not change during the defined grass pollen period (Fig. 10-2).

At the peak of grass pollen season (2nd June 2009), the EcN group had a SMS twice as much as the placebo group (EcN [median] = 8.5 points [1.0-17.0]; placebo [median] = 4.0 points [0.0-16.0]).

While the SMS of the EcN group increased with higher grass pollen counts, the SMS of the placebo group decreased steadily. Both groups had slightly decreased SMS values (EcN [median] = 4.0 points [0.0-17.0]; placebo [median] = 1.5 points [0.0-17.0]) at the end of the defined grass pollen season (3rd July 2009).

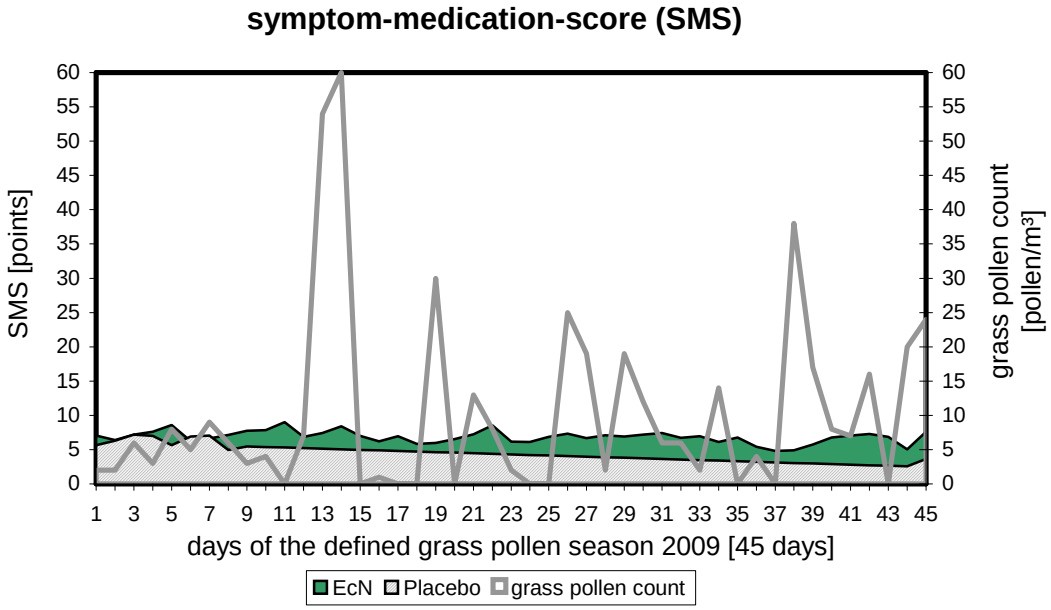

Fig. 10-2: Daily SMS of both treatment groups during the defined grass pollen season 2009 (45 days). The daily SMS over the 45 days result in the AUC.

For individual patient data listing of the SMS see appendix table 15.2.4.

10.2.1.2 Secondary efficacy parameters

Symptom score and medication score

The symptom score (SS) and medication score (MS) can be assessed simultaneously to the SMS as the SMS is the sum of both single scores.

The median sum of the SS of the EcN group over the defined grass pollen season (45 days) was higher compared to the placebo group (EcN = 160.5 points [40.0-541.0]; placebo = 110.0 points [14.0-431.0]). The median SS was 3.0 points (1.0-12.5) in the EcN group and 2.0 points (0.0-9.0) in the placebo group.

The median sum of the MS of the EcN group over the defined grass pollen season (45 days) was higher compared to the placebo group (EcN = 60.8 points [0.0-206.0]; placebo = 19.8 points [0.0-244.0]). The median MS was 1.0 points (0.0-4.0) in the EcN group and 0.0 points (0.0-5.0) in the placebo group.

For individual patient data listing of the SS and MS see appendix table 15.2.5 and 6.

Skin prick test (SPT)

The SPT with grass pollen extract was performed at every visit (see Tab. 13-1). In both treatment groups the results were similar and remained constant over the blinded EcN supplementation (Fig. 10-3).

In the EcN group the mean wheal diameter before the grass pollen season was 7.50 ±1.97 mm at screening, 7.40 ±3.00 mm at visit 2, and 6.17 ±1.93 mm at visit 3. During grass pollen

season (visit 4 and 5) the SPT values were 7.13 ±2.20 mm and 7.50 ±3.51 mm, respectively. At the follow-up the mean wheal diameter was 7.47 ±2.51 mm.

In the placebo group the mean wheal diameters were higher at each visit. These differences failed to be statistically significant. The mean wheal diameter was 8.25 ±2.23 mm and 8.07 ±2.01 mm at screening and visit 2, respectively. The mean wheal diameter at visit 3, 4 and 5 were 6.42 ±1.98 mm, 6.57 ±1.73 mm, and 7.75 ±1.74 mm, respectively. At the follow-up the mean wheal diameter was 7.27 ±1.18 mm.

In summary, no significant differences were detectable between before to after blinded EcN supplementation or between EcN and placebo.

For individual patient data listing of the SPT see appendix table 15.2.7.

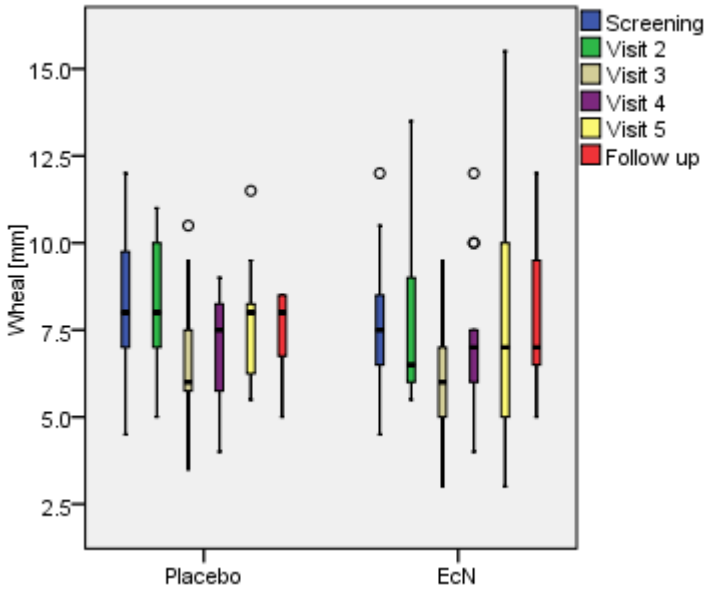

Fig. 10-3: Skin prick test (SPT) results during blinded EcN supplementation.

Conjunctival provocation test (CPT)

Similar to the SPT the CPT showed no significant differences between both treatment groups and in the start-end-comparison (see Tab. 10-1).

In the EcN group 1 CPT was negatively and 13 were positively before the blinded EcN supplementation. In 2 patients the CPT could not be performed. Among the positive reactions, 59% reacted at the lowest concentration (20.000 SQ/ml), 23% at 50.000 SQ/ml, and 8% at the highest concentration (100.000 SQ/ml).

In the placebo group the CPT was in 1 patient negatively and in 11 patients positively before the blinded EcN supplementation, it was not performed in 2 patients. Here, 64% of the positive patients reacted at the lowest concentration, 27% at the second concentration, and 9% at the highest concentration.

At the last visit, 12 patients of the EcN group reacted positively, 92% at the lowest concentration and 8% at the highest concentration. In 4 patients the CPT could not be performed. In the placebo group, 10 patients reacted positively, 70% at the lowest, 20% at the second, and 10% at the highest concentration. In 4 patients the CPT could not be performed. In comparison to visit 1, more positive reactions occurred at the lowest concentration, especially in the EcN group. However, the differences failed to be statistically significant.

Tab. 10-1: Number of patients for the conjunctival provocation test (CPT) before and after the blinded EcN supplementation.

CPT	before EcN supplementation		after EcN supplementation	
concentration	positive (EcN)	positive (Placebo)	positive (EcN)	positive (Placebo)
negative	1	1	0	0
20.000 SQ/ml	9	7	11	7
50.000 SQ/ml	3	3	0	2
100.000 SQ/ml	1	1	1	1
Not done	2	2	4	4
total	16	14	16	14

For individual patient data listing of the CPT see appendix table 15.2.8.

Specific IgE

Specific IgE was measured in serum at screening and visit 5. In the EcN group the median sIgE value was 14.6 kU/l (1.1-100.0) at screening and 14.2 kU/l (0.9-100.0) at visit 5. In the placebo group the median sIgE value was 17.8 kU/l (8.4-100.0) at screening and increased to 24.1 kU/l (8.7-100.0) at visit 5. This change was not statistically significant.

For individual patient data listing of the sIgE see appendix table 15.2.9.

Quality of life

To assess the quality of life before, during and after the blinded EcN supplementation and grass pollen season, the patients received the rhinoconjunctivitis quality of life questionnaire (RQLQ, Version with standardised activities ©2007) at every visit (see Tab. 13-1). The RQLQ was analysed according to Elisabeth F. Juniper (Juniper 1996 JACI “Interpretation of RQLQ data”). The questions are divided into 7 domains: activities (3 questions), sleep (3 questions), non-hayfever symptoms (7 questions), practical problems (3 questions), nasal symptoms (4 questions), eye symptoms (4 questions), and emotions (4 questions). The result is expressed as the mean score per domain as well as for overall quality of life. Thus, the domain and overall scores range from 0 to 6.

As the RQLQ is collected at different time points, it is possible to judge whether a particular change in score represents an important improvement or deterioration, or whether it represents a trivial change. A change of -1/0/+1 points are considered as trivial changes. Changes of +2 or greater and -2 or less are important to patients, thus, considered as clinically important differences.

During the clinical trial both groups showed a grass pollen season depending course of life quality. However, both groups had no noteworthy changes in the RQLQ score. In both treatment groups mostly trivial changes occurred (-1/0/+1). Thus, the overall life quality influenced by the rhinoconjunctivitis was very good in the investigated population during the grass pollen season 2009.

In the EcN group the median RQLQ score was 0.29 points (0.00-2.37) and 0.34 points (0.00-3.46) before and after the blinded EcN supplementation, respectively (Fig. 10-4). In comparison, the placebo group started with a median RQLQ score of 0.93 points (0.00-2.85) and decreased to 0.27 points (0.00-1.60) at visit 5, but failed to be statistically significant.

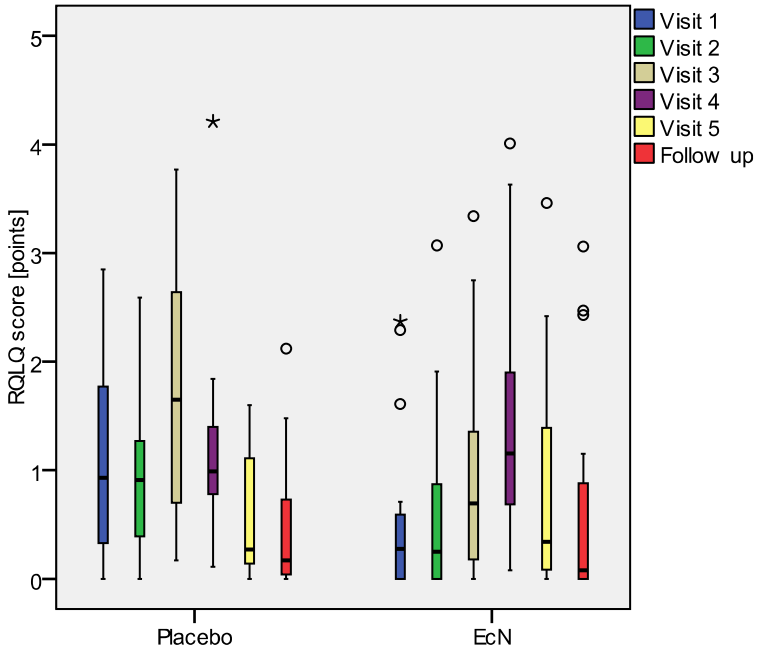

Fig. 10-4: Quality of life before, during and after blinded EcN supplementation. Data are depicted as mean RQLQ score.

For individual patient data listing of the RQLQ score see appendix table 15.2.10.

Symptom-free days

Symptomfree days were defined as days without the use of allergic medication and a symptom score ≤2 points. These days calculated by the SS and MS over the defined grass pollen season (45 days). The median number of symptom-free days in the EcN group was lower compared to the placebo group (EcN = 6 days [0-40]; placebo = 18 days [0-44]). However, this difference was not statistically significant.

For individual patient data listing of symptom-free days see appendix table 15.2.11.

Global evaluation of the grass pollen season 2009

Finally, the patients were asked if and how their rhinoconjunctivitis symptoms changed compared to the previous season 2008 at the last visit (visit 5, see Fig. 10-5).

In the EcN group 13% of the patients stated that the treated grass pollen season 2009 was much better, 38% evaluated it as better, and 38% as the same compared with the previous grass pollen season (2008). Only two patients evaluated the treated season worse than the previous one.

In the placebo group 14% of the patients said the treated grass pollen season 2009 was much better, 36% evaluated it as better, and 14% as the same compared with the previous grass pollen season (2008). More patients (29%) evaluated the treated season as worse compared to the previous season.

In both groups, no patient evaluated the treated grass pollen season as much worse than the previous season.

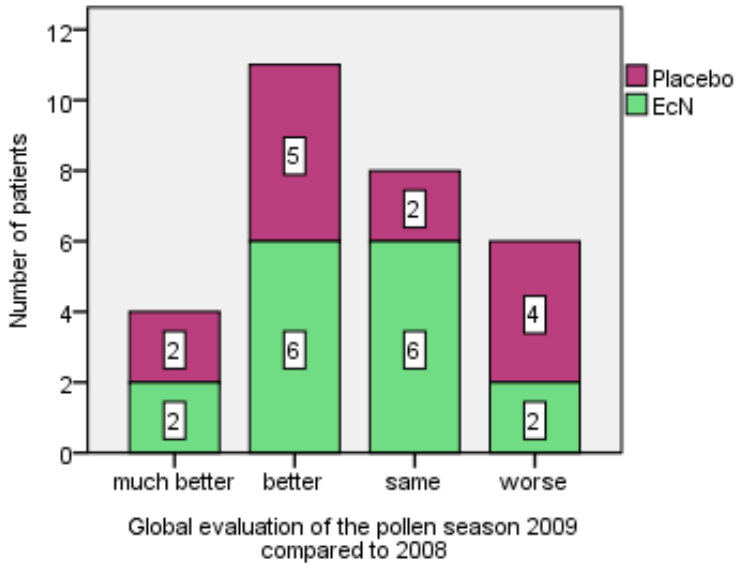

Fig. 10-5: Global evaluation of rhinoconjunctivitis symptoms during the treated grass pollen season 2009 compared to 2008.

For individual patient data listing of global evaluation of rhinoconjunctivitis symptoms see appendix table 15.2.12.

10.2.2 Efficacy conclusion

The primary efficacy parameter, SMS failed to be statistically significant different between placebo and EcN group. Thus, our hypothesis, that EcN has immunmodulatory capacities and consequently reduces the clinical symptoms of patients suffering from AR to grass pollen could not be confirmed. Already at the first day of defined grass pollen season, the SMS score was higher in the EcN group and was never below the SMS core of the placebo group during the defined grass pollen season (45 days). At the peak of grass pollen season, the EcN group had a SMS twice as much as the placebo group. However, the SMS of the EcN group changed in connection to the grass pollen count, which indicates that the investigated group treated with EcN had rhinoconjunctivitis symptoms surely depending on the grass pollen count. In contrast, the SMS of the placebo group decreased steadily. Here, it should be considered that the rhinoconjunctivitis symptoms mainly not depending on the grass pollen count.

Although, the secondary efficacy parameter did not show any significant differences between placebo and EcN, some parameters were different among the groups. In the EcN group more patients reacted in the CPT at the lowest allergen concentration after the grass pollen season (11 patients). No changes were seen in the placebo group. This may suggests a higher sensitivity in the EcN group.

Regarding the global evaluation, more patients in the EcN group assessed their symptoms as the same compared to the previous grass pollen season (EcN: 6 patients versus Placebo: 2 patients). However, only 2 patients in the EcN group experienced a worsening of symptoms compared to 4 patients in the placebo group.

11 Safety evaluation

11.1 Adverse Events

11.1.1 Brief summary of adverse events

AEs were observed in 162 (ITT) and 144 (PP) cases. In 18.52% (ITT) of these cases was a possible connection to the trial medication documented.

No AE was defined as severe in connection to the trial medication. One AE was definitely related to EcN. Most events with a possible relationship to EcN were diarrhoea (6 of 162 AEs), abdominal pain (6 of 162 AEs) and flatulence (8 of 162 AEs). Those AEs were recovered within a few days.

Two possible treatment-related AEs occurred at the end of the clinical trial and after stopping the daily intake (p-no: 2, p-no: 7) with similar gastrointestinal symptoms like at the beginning of the treatment.

One AE, which was not treatment-related, leads to discontinuation of the supplement for three days but was continued after clinical examination by the investigator (p-no.11).

No SAE was observed and no death entered.

Most frequently observed symptoms in non-trial medication related AEs were headache (39 of 162 AEs) and cold (30 of 162 AEs). The AEs were mainly mild (96 of 162 AEs) or moderate (65 of 162 AEs) in intensity.

11.1.2 Display of general adverse events

Tab. 11-1: General adverse events: Summary of concerned system organ classes

All treatment-emergent adverse events	**ITT group total 34 patients**	
System organ class	**F**	**N**
Musculoskeletal and connective tissue disorders	9	9
Cardio-vascular disorders	41	19
Skin, mucosa and subcutaneous tissue disorders	6	4
Nervous system disorders	3	3
Ophthalmic disorders	4	3
Endocrinal disorders	2	1
Respiratory, thoracic and mediastinal disorders	46	24
Urogenital disorders	2	2
Gastrointestinal disorders	49*	20
TOTAL	**162**	**85**

Source: Appendix Table 15.2.13
F = number of adverse events, N = number of patients with adverse events
* 31 were possible or sure related to EcN

Tab. 11-2: General adverse events: Summary of characteristics

		ITT group total 34 patients	
Category		**F**	**N**
Related	No	131	9
	Possible	30*	10
	Yes	1*	1
TOTAL		**162**	**20**
Intensity	mild	96	28
	moderate	65	25
	severe	1	1
TOTAL		**162**	**54**
Outcome	recovered	152	33
	ongoing	5	4
	stabilised	4	4
	sequelae	0	0
	patient died	0	0
	unknown	1	1
TOTAL		**162**	**42**
Action taken – concerning trial medication	treatment unchanged	158	33
	drug reduced	0	0
	permanently discontinued	3	2
	temporarily discontinued	1	1
TOTAL		**162**	**36**
Action taken – other	medication	79	31
	none	83	4
	other	0	0
TOTAL		**162**	**35**

Source: Appendix Table 15.2.13
F = number of AEs, N = number of patients with AEs
* only gastrointestinal AEs

11.1.3 Display of EcN-related adverse events

Tab. 11-3: EcN-related AE in the EcN group

AEs possible related to EcN	**ITT EcN group (16 patients)**	
characteristics	**F**	**N**
Diarrhoea	7	5
Abdominal pain	6	5
Flatulence	9*	7*
Nausea	4	4
Others (opstipation)	1	1
TOTAL	**27**	**22****

Source: Appendix Table 15.2.13
F = number of EcN-related AEs, N = number of patients with gastrointestinal-related AEs
* In one patient flatulence was surely related to EcN and the treatment was stopped (p-no. 30).
** Some patients experienced more than one gastro-intestinal symptom which was related to EcN. In total, 8 patients experienced EcN-related AEs.

Tab. 11-4: EcN-related AEs in the placebo group.

AEs possible related to EcN	ITT placebo group (16 patients)	
characteristics	**F**	**N**
Diarrhoea	3	3
Abdominal pain	6	2
Flatulence	4	3
Nausea	0	0
Others (stomach cramp)	1	1
TOTAL	**14**	**9****

Source: Appendix Table 15.2.13
F = number of gastrointestinal-related AEs, N = number of patients with gastrointestinal-related AEs
** Some patients experienced more than one gastro-intestinal symptom which was related to EcN. In total, 5 patients experienced EcN-related AEs.

11.1.4 Listing of adverse events by patient

All AEs are listed by patient in appendix table 15.2.13.

11.1.5 Analysis of adverse events

Overall, mainly mild AEs in both treatment groups occurred (Tab. 11-2). AEs assigned to the cardio-vascular system, the respiratory, and thoracic or mediastinal compartment and the gastrointestinal tract were the most common events (Tab. 11-1). Headache was one of the most frequent AE. Only one AE (flatulence, p-no. 30) could be surely related to EcN. The AEs which were valued as possible related to EcN were all located in the gastrointestinal tract. The patient (p-no. 30) felt too uncomfortable and wished to stop the treatment. Except of this AE all other AEs had no effect on the study treatment or on the evaluation of treatment efficacy.

11.1.6 Discontinuation/pretermination due to adverse events

One patient (p-no. 30) stopped the clinical trial prematurely due to an AE. Symptoms were severe flatulence. The patient stopped the intake of trial medication and withdrew the participation. During a last examination no symptoms were reported and recovered within 1 day after stopping the intake of trial medication.
One patient (p-no. 9) discontinued the intake for 16 days due to an AE (No. 4, Cimikosis). The onset of the AE was not related to the trial medication by the investigator.

11.2 Death, other serious adverse events and other significant adverse events

No SAEs or to death leading events occurred. No other significant AEs were reported that shows newly appeared reactions of the trial treatment.

11.3 Clinical laboratory Evaluation

11.3.1 Listing of individual laboratory measurements by patient and each abnormal laboratory value

Individual laboratory values are listed in appendix table 15.2.15.

11.3.2 Evaluation of each laboratory parameter

The safety laboratory was done only before blinded EcN supplementation. There was no indication to control the laboratory parameters during or after the end of trial for any patient. Overall, 4 measurement were above the normal range and 5 below the normal range. Those 9 measurements were valued to be not clinical relevant and are marked in the appendix table 15.2.15.

11.4 Vital signs, physical findings, and other observations related to safety

11.4.1 Listing of individual vital signs, physical findings and other observations

Individual vital signs, physical findings and other observations are listed in appendix table 15.2.16 and 17.

11.4.2 Evaluation of each vital parameter

Vital parameters and physical findings were checked and controlled during the screening and the last visit (visit 5) and during other visits if needed. There were two indications to control the vital parameters during the clinical trial (p.-no: 33 and 11) given because both patients had adverse events which leaded to unscheduled visits.

Overall, 7 abnormal physical findings have been found during the screening but none of them changed until the end. Those 7 observations were valued to be not clinical relevant and are specified in the appendix table 15.2.17.

11.5 Evaluation of tolerability

The tolerability of trial medication was assessed after the blinded EcN supplementation at visit 5 by the patient and by the investigator on a 4-point scale (1 – very good, 2 – good, 3 – moderate, 4 – bad). The tolerability of EcN was evaluated “very well” by the majority of the treated patient as well as by the investigator. In the placebo group the majority assessed the tolerability mainly with “well”. However, the overall tolerability was good both groups.

Individual patients listing of tolerability see appendix table 15.2.19.

11.6 Safety conclusion

The used IMP (Mutaflor®) is already known and used as medication for years with a low rate of adverse events. The EcN-related AEs were all located in gastrointestinal tract and mainly mild in its intensity. It is known that these symptoms (flatulence, abdominal pain, diarrhoea) might occur in the beginning Mutaflor® intake, but resolve by when the patient got used to it. In general, the majority of AEs were not related to the trial medication. Thus, the blinded EcN supplementation was safe and well tolerated.

12 Discussion and overall conclusion

Animal experiments and in vitro studies showed a promising immunmodulatory effect of EcN (see protocol chapter 3.1.3 and 3.1.4). However, the assumed immunmodulatory effect of EcN was not confirmed in this clinical trial with patients suffering from AR to grass pollen.

Some problems for the failed statistically significant different between placebo and EcN will be discussed here. One limitation of pilot studies, in general, is the small sample size which is investigated. Although the patient groups seem to be very homogenous (Tab. 13-2), differences were detected in the total and specific IgE as well as in the SS at the beginning of grass pollen season (20th May 2009, chapter 10.2.1.2).
As the majority of patients were not mono-sensitised, one can speculate that the beginning of EcN supplementation was too late. Most patients suffered from birch pollen allergy as well so that the immune system was already triggered by birch pollen allergens. Thus, one may consider in further clinical trials a longer wash-in phase e.g. starting in winter.
The 1-season-investigation is suited for such a pilot study, as the results can be generated very fast, the compliance of patients is better and the costs can be controlled. On the other hand, as the primary efficacy parameter is depending ion the grass pollen count, the outcome of the clinical trial is strongly influenced by environmental factors which determine the

current grass pollen count. An investigation over 2 or 3 grass pollen seasons might be more appropriate.
The allergen concentrations which we chose for the CPT might be not appropriate to detected differences between both groups. The threshold of eliciting symptoms could not be proper determined with only 3 steps. In further studies an allergen titration with more steps e.g. 100, 330, 1.000, 3.300, 10.000, 33.000 and 100.000 SQ-U/ml should be considered.
However, if present, the immunmodulatory effect of EcN is weak if it is applied as a single drug. Whether the combination with e.g. specific immunotherapy may be more promising should be investigated in further studies.

13 Figures and tables referred to but not included in the text

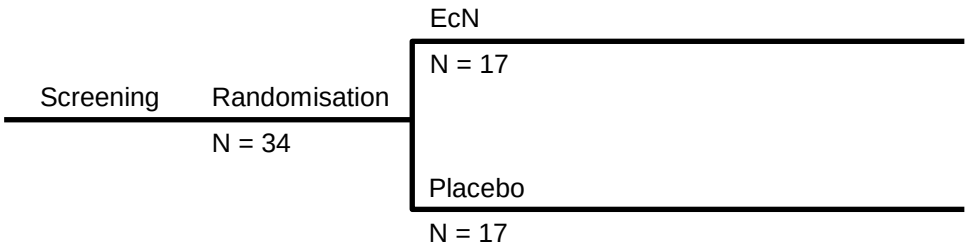

Fig. 13-1: Treatment groups, two-armed parallel design. EcN – *Escherichia coli* Nissle 1917

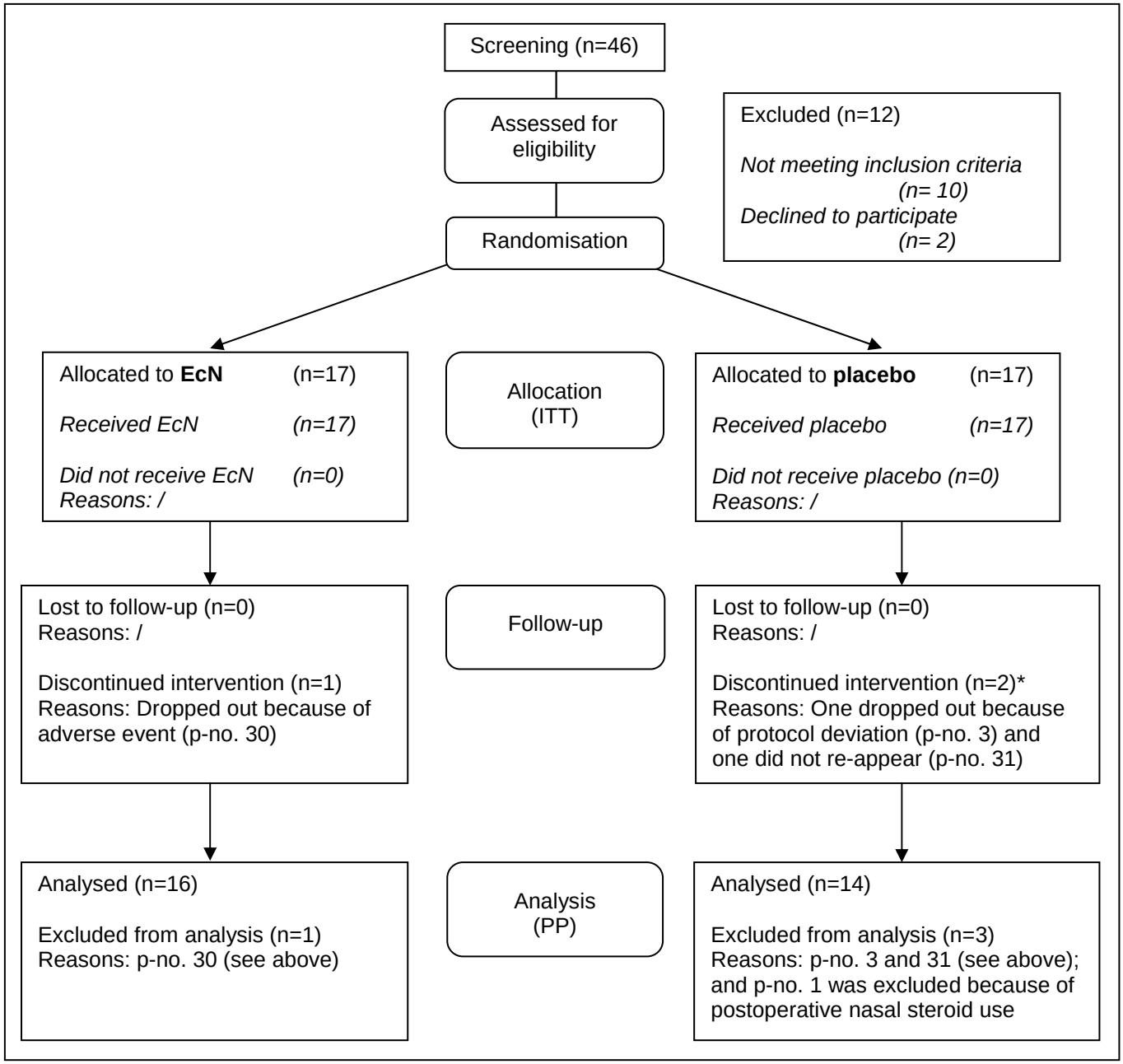

Fig. 13-2: CONSORT Flow Diagram. According to Schulz et al. 2010 [7]. ITT – intention-to-treat, PP – per-protocol, *one patient (p-no. 11) dropped out, but the complete data set for the primary efficacy parameter was present, thus, this patient could be included in the efficacy analysis.

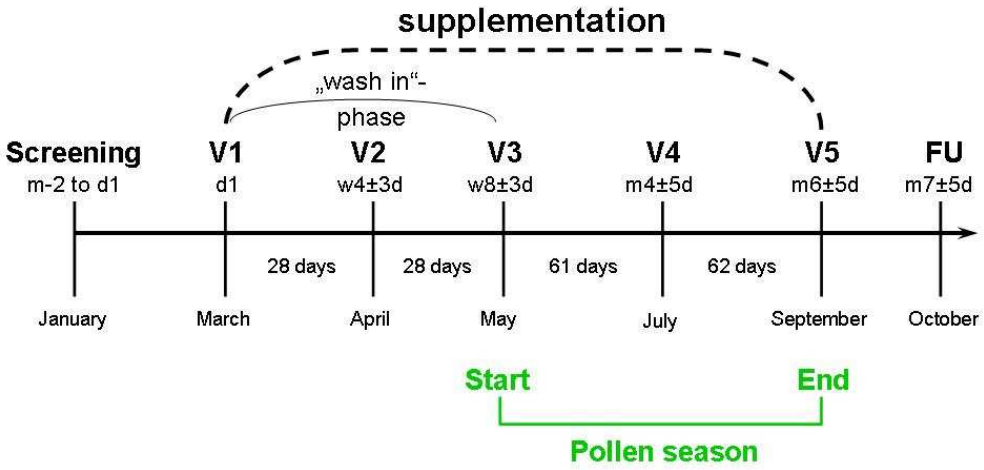

Fig. 13-3: Time schedule of the clinical trial in general.

Tab. 13-1: Time schedule of the trial procedures.

treatment	**Screen-ing**	**Visit 1**	**Visit 2**	**Visit 3** [v] start pollen season	**Visit 4** [v]	**Visit 5**	**Follow-up**
day, week, month	m-2 to d1	d1	w4 ± 3d	w8 ± 3d	m4 ± 5d	m6 ± 5d	m7 ± 1w
Patient information	✓						
Informed consent	✓						
Clinical history	✓						
In- and exclusion criteria	✓						
Ethical origin	✓						
Body height and weight	✓						
Vital signs[#]	✓	i.n.	o.d.	o.d.	o.d.	✓	o.d.
Physical examination	✓	i.n.	o.d.	o.d.	o.d.	✓	o.d.
Pregnancy test[##]	✓	i.n.					
Lung function test (FEV_1)	✓						
Skin prick test (SPT)*	✓	i.n.	✓	✓	✓	✓	✓
Conjunctival provocation test		✓				✓	
IgE, sIgE	✓					✓	
Safety lab	✓						
Immune cell measurement		✓		✓		✓	✓
Dispense of patient's diary		✓	✓	✓	✓		
Control of patient's diary**			✓	✓	✓	✓	
Return of patient's diary**			✓	✓	✓	✓	
SMS°			✓°	✓°	✓°	✓°	
rSS***	✓					✓	
Life quality questionnaire		✓	✓	✓	✓	✓	✓
Dispense of trial medication		✓	✓	✓	✓		
Control of trial medication			✓	✓	✓		
Return of trial medication						✓	
Dispense of rescue medication[∞]			✓				
Return of rescue medication						✓	

Start of supplementation		✓					
End of supplementation						✓	
Documentation of tolerability			✓	✓	✓	✓	
Documentation of AE		✓	✓	✓	✓	✓	✓
Documentation of ConMed	✓	✓	✓	✓	✓	✓	✓

AE – adverse event, ConMed – concomitant medication, i.n. – if necessary, o.d. – on demand

v An additional appointment to change patient's diaries is planned four weeks after visit 3 and visit 4 (visit 3a/visite 4a).

Vital signs are blood pressure and heart beat.

for woman with child bearing potential

* Eight allergens are applied at screening and only grass is applied at all the other visits.

** Change of patient's diary is planned monthly, therefore two additional visits are needed (visit 3a/visite 4a).

*** rSS – retrospective symptom score: The patient is asked to score his rhinoconjunctivitis symptoms during the grass pollen season of 2008 at screening. The patient is asked to score his rhinoconjunctivitis symptoms during the grass pollen season of 2009 (supplemented season) at visit 5. Herein the time with the most severe symptoms should be considered.

° SMS – symptom-medication-score is assessed within the diary daily.

∞ The use of rescue medication is assessed within the diary.

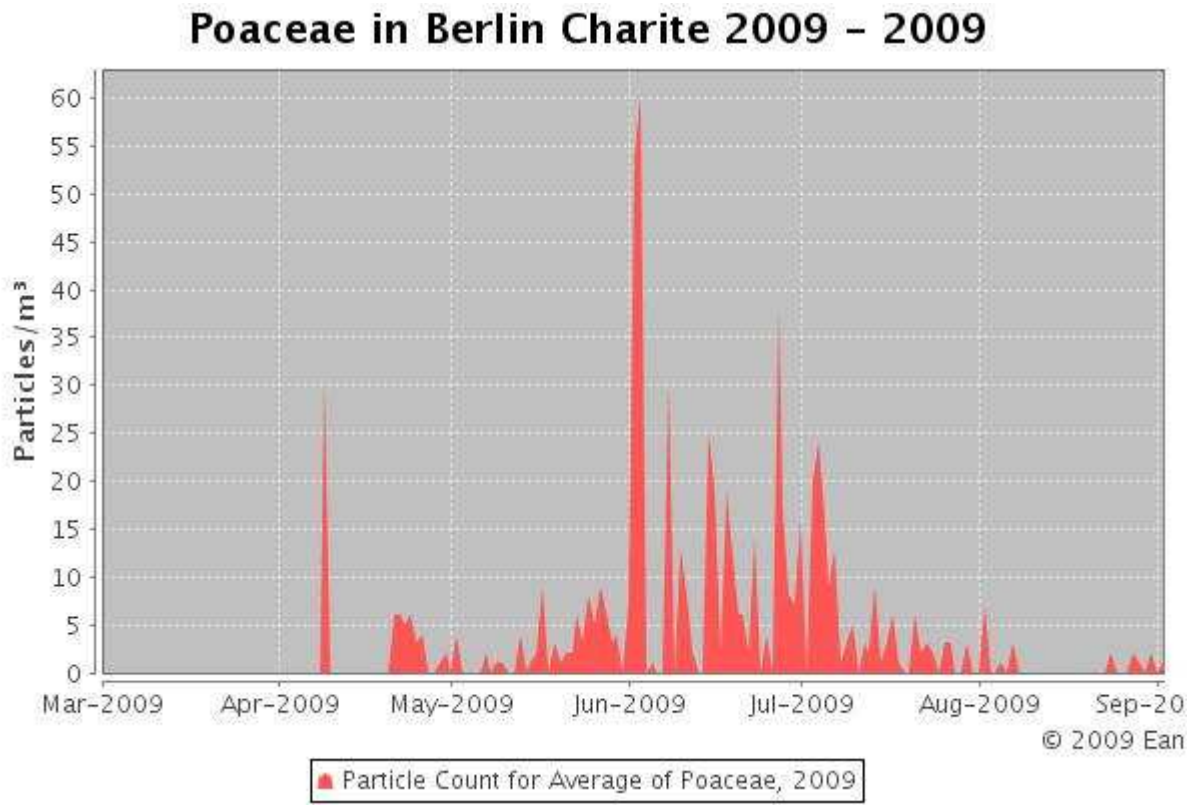

Fig. 13-4: Grass pollen count in Berlin, Germany 2009. The figure is kindly provided by the foundation of pollen information service (Stiftung Deutscher Polleninformationsdienst).

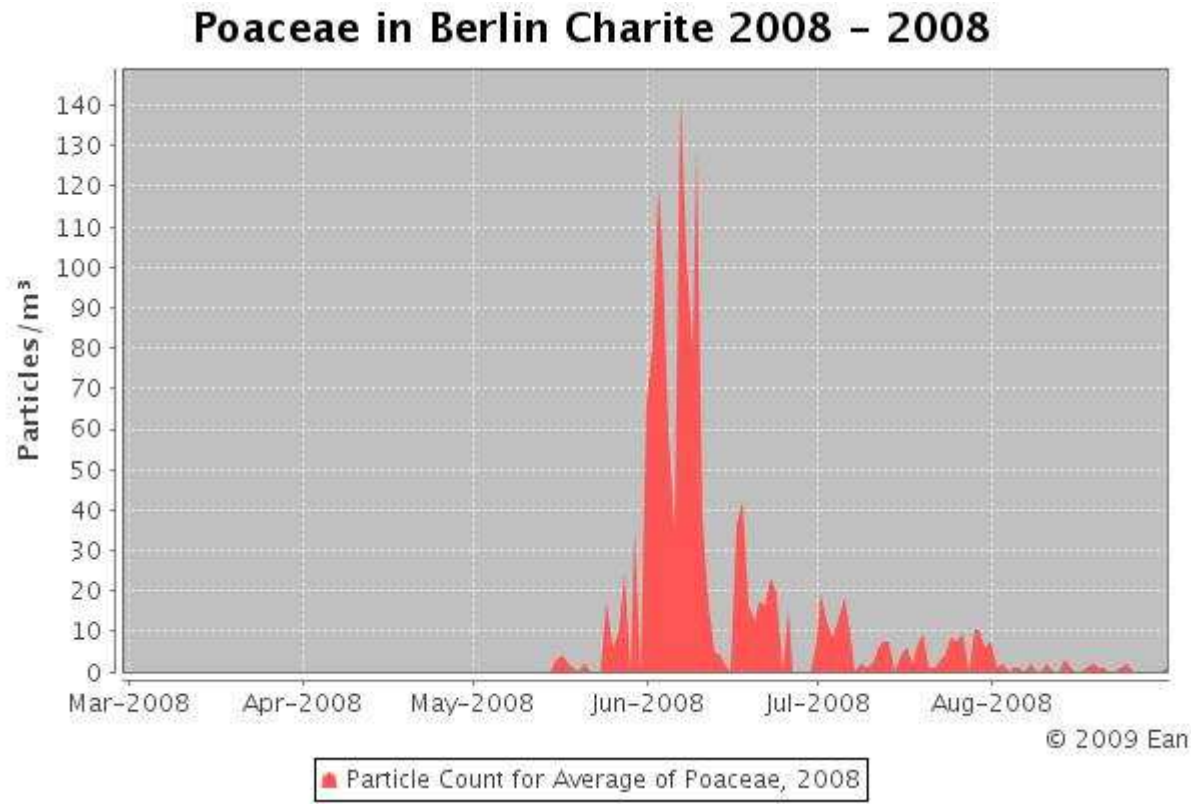

Fig. 13-5: Grass pollen count in Berlin, Germany 2008. The figure is kindly provided by the foundation of pollen information service (Stiftung Deutscher Polleninformationsdienst).

Tab. 13-2: Demographic data

	Before blinded EcN supplementation	
characteristics	**EcN, N=17**	**Placebo, N=17**
female / male	12 / 5	8 / 9
age (years)	35 [20-54]	36 [19-49]
sIgE (kU/l)	14.4 [1.1-100.0] 1 patient >100.0	22.9 [8.4-100.0] 1 patient >100.0
total IgE (kU/l)	96.0 [16.0-383.0]	252.0 [28.4-1417.0]
SPT (mm)	7.0 [4.5-12.0]	7.5 [4.5-12.0]
rSS (points)	17 [12-21]	17 [12-21]
RQLQ (points)	0.29 [0.00-2.37]	0.93 [0.00-2.85]
Safety parameters ALT (U/l) AP GGT	 24.1 ±8.1 60.4 ±19.4 19.8 ±14.7	 24.9 ±16.3 56.2 ±13.9 23.2 ±19.0

14 Reference list

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