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Influence of Tiopronin on the Metabolism of Alcohol in Healthy Subjects

Dissertation

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ABSTRACT

Background: Many drug safety- and drug-alcohol interaction studies have been done for frequently used medications or drugs with high financial interests. Orphan drugs like tiopronin – a drug which is used in the treatment of the rare disease cystinuria - are often neglected in terms of clinical research. We analysed the interaction of tiopronin on the metabolism of alcohol and the medication's safety in this combination in healthy volunteers.

Methods: In this investigator-driven randomised, double blind, cross-over study, 13 healthy subjects received the medication (500 mg tiopronin or an identical looking placebo) one hour before the intake of 0.8 g alcohol per kg bodyweight. The two study days, in which each participant received each treatment once, were one week apart. For the primary objective blood alcohol concentrations were measured 0 h (before intake of alcohol, after intake of study drug), 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h and 12 h after the consumption of alcohol. Primary objectives AUC and elimination rate k were analysed based on a mixed linear model with mixed treatment-effect, fixed period-effect and fixed interaction term. Secondary objectives such as C_{max}, t_{max} values of alcohol metabolism, acetaldehyde blood levels, concentration abilities and the drug's safety profile were determined with similar analysis and descriptive statistics.

Results: There was no significant treatment effect for either of the objectives. Significant differences in periods could be found in concentration tests and seem to be based on learning effects. No serious adverse event (SAE) occurred. All adverse event were reversible and similarly distributed among none treatment and treatment.

Conclusion: It was shown that tiopronin does not affect the metabolism of alcohol. There were no SAE when combining tiopronin and alcohol in healthy subjects [1].

ZUSAMMENFASSUNG

Hintergrund: Gewinnbringende und häufig verwendete Medikamente sind sehr gut erforscht. In Bezug auf die Arzneimittelsicherheit, Interaktionen mit anderen Medikamenten und Interaktionen mit Alkohol liegen viele Studienergebnisse vor. Selten verwendete Medikamente wie Tiopronin, eine Substanz, die seit Jahrzehnten vor allem in der Behandlung der Cystinurie eingesetzt wird, werden auf diesem Gebiet häufig vernachlässigt. Diese Studie untersuchte den Einfluss von Tiopronin auf den Metabolismus von Alkohol und die Sicherheit dieser Kombination bei gesunden Freiwilligen.

Methoden: In dieser von der Untersucherin durchgeführten randomisierten, doppel-verblindeten cross-over Studie erhielten 13 gesunde Probanden die Studienmedikation (500mg Tiopronin oder rein identisch aussehendes Placebo) eine Stunde bevor sie 0,8g Alkohol pro kg Körpergewicht zu sich nahmen. Es gab insgesamt zwei Studientage im Abstand von einer Woche, an denen jeder Teilnehmer jeweils einmal Placebo und einmal Tiopronin erhielt. Für die primäre Zielgrößen wurden Blutalkoholkonzentrationen zum Zeitpunkt 0 h (vor der Einnahme von Alkohol und nach der Einnahme der Studienmedikation), 15 min, 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h und 12 h nach Alkoholeinnahme gemessen. Die AUC der Konzentrations-Zeitkurve sowie die Eliminationsrate k wurden mit Hilfe eines gemischt linearen Models ausgewertet. Es wurden Behandlungseffekte, Periodeneffekte und Interaktionen berechnet. Sekundäre Zielgrößen wie C_{max} und t_{max} der Alkoholkurven sowie Blutacetaldehydwerte, Konzentrationsfähigkeiten und Sicherheitsmessungen wurden mit ähnlichen statistischen Methoden und deskriptiver Analyse verglichen.

Ergebnisse: Für keine der untersuchten Zielgrößen konnte ein signifikanter Behandlungseffekt gemessen werden. Die beobachteten signifikanten Verbesserungen in den Ergebnissen des Konzentrationstests zwischen und innerhalb von Perioden können mit einem Lerneffekt erklärt werden. Die Behandlung mit Tiopronin hat keine schweren Nebenwirkungen (SAE) hervorgerufen. Alle auftretenden Nebenwirkungen waren reversibel und erschienen gleichmäßig verteilt nach Placebo- und Tiopronin-Einnahme.

Schlussfolgerung: Tiopronin hat keinen Einfluss auf den Metabolismus von Alkohol. Es gab keine SAE unter der Kombination Tiopronin und Alkohol bei gesunden Freiwilligen [1].

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADH	alcohol dehydrogenase
AE	adverse event
AUC	area under the curve
BAC	blood alcohol concentration
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte/ The Federal Institute for Drugs and Medical Devices
BMI	body mass index
BrAC	breath alcohol concentration
BP	blood pressure
BSG	Blutsenkungsgeschwindigkeit/ ESR= erythrocyte sedimentation rate
CK	creatine kinase
C_{max}	peak plasma concentration
CRF	case report form
CRP	C - reactive protein (acute phase protein)
ECG	electrocardiogram
EDTA	ethylene-diamine-tetraacetic acid, added to blood collection tubes
ESR	erythrocyte sedimentation rate
ESWL	extracorporeal shockwave lithotripsy
FPM	first pass metabolism
γGT	gamma-glutamyl transferase
GOT	glutamat-oxalacetat-transaminase
GPT	glutamat-pyruvat-transaminase
HR	heart rate
INR	international normalized ratio
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
MCHC	mean corpuscular haemoglobin concentration
MEOS	microsomal ethanol oxidising system
O2 %	blood oxygen saturation in %
SAE	serious adverse event

SPC	summary of product characteristics
t_{max}	time when the maximum plasma concentration is reached
TMF	trial master file
ZVT	Zahlenverbindungstest/ number-connection test

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1 INTRODUCTION

1.1 THE DRUG TIOPRONIN

Tiopronin is a reducing and complexing thiol compound with a molecular weight of 163.20. The substance occurs in white water soluble particles.

Also known as (N-(2-mercaptopropionyl)glycine), it exists as a dl-racemic mixture and has the following structure:

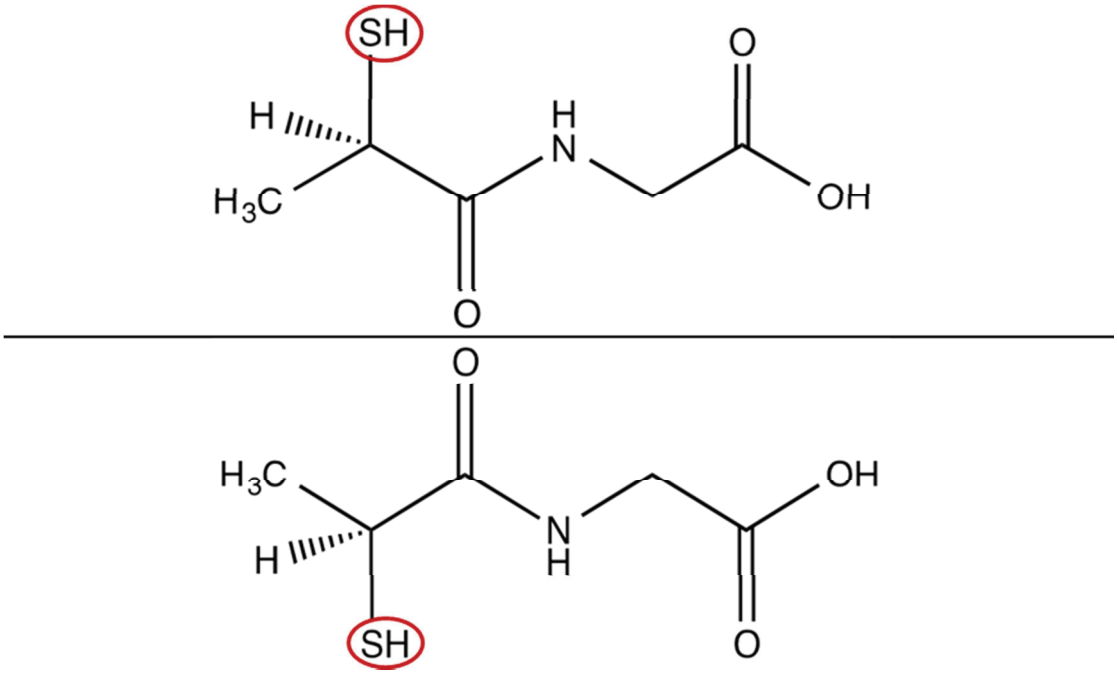

Fig. 1: Chemical structure of tiopronin

Due to its chelating and reducing character, tiopronin is used in different contexts. Tiopronin was firstly developed in the 1960's due to the Minamata disease (mercury intoxication) [2] and it is still used for mercury and other metal intoxications [3], [4]. With its thiol group, tiopronin is also able to neutralise reactive oxygen species [5] and works as anti-rheumatic drug [6]. It neutralizes toxic aldehydes in the brain and may have a neuroprotective role after cranial cerebral trauma [7], [8]. Moreover, this drug seems to have a function as radical scavenger after radiation or chemotherapy [9], [10], but the main indication of tiopronin today is the treatment and prophylaxis of medical complications due to cystinuria [1], [4], [11], [12].

1.2 THE DISEASE CYSTINURIA

Cystinuria is a lifelong hereditary disease, in which an amino acid transporter, responsible for the reuptake of specific amino acids from the primary urine, is defect. The disease has its origin in mutations in the SLC3A1 and SLC7A9 genes. These genes encode sections of a transporter protein that occurs primarily in the kidneys. Defects prevent proper reabsorption of cystine and basic, or positively charged amino acids such as lysine, ornithine, and arginine with the result that these amino acids accumulate in the primary urine. However the main problem for patients is the low water solubility of cystine resulting in stone formation in the urinary tract [1], [13].

Cystinuria appears equally in both genders, but symptoms are more frequently and fatal in male patients. Most patients experience the first stone between eleven and thirty years [14]. The prevalence of the disease is about 1:20.000 people worldwide [13] , but depends on ethnic groups; Israelic Jews coming from Libya have a prevalence of 1: 2.500 [1],[15].

Clinical symptoms of cystinuria are equal to kidney and bladder stones of other origins. Currently there is no curative therapy for cystinuria, but different methods to prevent and dissolve stone formations have been developed: the treatment starts with low salt intake and high hydration. Moreover the pH is kept slightly alkaline. If these methods are not sufficient a therapy with thiol compounds such as tiopronin or D-penicillamine will be started. Depending on the size of the stones and clinical symptoms, urological interventions such as ESWL, retrograde endoscopic lithotripsy and extraction and percutaneous nephrolithotomy are necessary [16].

Initially Cystinuria was described by Wollaston in 1810 and Berzellius a few years later [17], [18]. It took another hundred years before Tabachnick et al. [19] accidently discovered an interaction process between penicillamine and cysteine, so that Crawhall et al. treated the first patient suffering from Cystinuria with penicillamine in the 1960's [20].

However, D-penicillamine treatment is frequently accompanied by adverse skin and autoimmune reactions [21]. Therefore tiopronin may have a particular therapeutic role especially in these patients because of a lower rate of adverse events and side effects compared to D-penicillamine [1].

1.3 TIOPRONIN IN CYSTINURIA

In the early 1970s tiopronin was introduced in the treatment of cystinuria [12].

The drug tiopronin forms a thiol-disulfide bond with cysteine:

$$2\,R-SH \quad + R'\text{-}S\text{-}S\text{-}R' \rightarrow 2\,R-S-S-R' \quad + 2\,H+$$

$$\text{tiopronin} \quad + \text{cystine} \rightarrow \text{tiopronin}-\text{cysteine} \quad + 2\,H+$$

From this reaction, the water-soluble mixed disulfide tiopronin-cysteine is formed and eliminated via urine, and the amount of sparingly soluble cystine, which causes the symptoms of cystinuria (pain due to kidney stones), is reduced [12]. According to the Marketing Authorisation Holder, sales figures for “Captimer” (brand name for tiopronin in Germany) suggest that about 400 patients were treated with the drug on a regular basis in Germany in the year 2015 [1].

1.4 RATIONALE FOR CONDUCTING THE STUDY

According to the SPC for "Captimer", it is not allowed to drink alcohol while using this drug [4], due to lack of availability of information. But cystinuric patients being on a lifelong daily medication may not be compliant during their whole life, making it necessary to obtain more evidence about the interaction of tiopronin and alcohol. There have no clinical studies been found addressing this topic (see 3.1.2) [1].

For penicillamine, a medication with partially identical indications and a similar chemical structure, no interaction with alcohol is known [22].

However D-penicillamine seems to be an effective sequestration agent for acetaldehyde, the first metabolic product in oxidative pathways of ethanol elimination. Lowering acetaldehyde levels by taking D-penicillamine may reduce adverse events of alcohol consumption and addiction [23], [24]. D-penicillamine has more frequently and stronger side effects than tiopronin [25] (see 1.2), therefore the influence of tiopronin on the

analysis of blood acetaldehyde levels and possible changes in concentration abilities were included as objectives in this study [1].

Based on one animal study, there is information available illustrating that tiopronin, when used about 1 hour before the application of alcohol, may reduce the alcohol concentration to blood in a great extent [25].

To elucidate this apparent contradiction, a study examining the influence of tiopronin on the metabolism of alcohol in healthy human beings was performed [1].

1.5 METABOLISM OF ALCOHOL

1.5.1 General considerations

Widmark firstly described the pharmacokinetics of alcohol. He was a pioneer in the implementation of standardized clinical trials; creating concentration-time profiles of ethanol, which are still relevant in forensic and clinical every day work [26].

Widmark measured alcohol in g/kg in whole blood. Most results in alcohol research are now reported in mass/volume and rho in L/kg [27].

Today samples are often analysed in serum, but published papers refer to serum and whole blood data. The following table 1 summarises the common used units for blood ethanol concentrations in different nations [28], [29]:

Unit	Area of application
mg/g = ‰	Norway, Sweden, Germany
mg/ml	Many other European countries (e.g. Austria, The Netherlands)
mg/100ml	Great Britain, New Zealand
g% (weight/volume), g/100 ml	United States of America, Australia, Canada
mmol/l (100mg/dl = 21.7 mmol/l)	Biomedical research

Table 1: Blood alcohol units [28], [29]

The laboratory involved in this study (central laboratory Goch) conveyed values in ‰ in serum. The values can be divided by 1.2 to convert the results in ‰ whole blood using the current forensic German guidelines [30].

$$BAC(‰\ in\ whole\ blood) = \frac{ethanol\ in\ serum\ (‰)}{1.2}$$

Equation 1: Conversion BAC serum in whole blood

The density of serum has to be considered as well, if samples measured in mg/ml are converted in mg/g = ‰. The density of serum is 1.03 (g/ml) [30].

$$BAC(‰\ in\ whole\ blood) = \frac{ethanol\ in\ serum\ (\frac{g}{ml})}{1.2\ \times 1.03\ (\frac{g}{ml})}$$

Equation 2: Conversion BAC serum (g/ml) in whole blood (‰)

The administration, absorption, distribution and elimination of alcohol determine measurements of its blood concentrations. The following lines try to sum up the fate of alcohol in the human body.

1.5.2 Administration and Absorption

Over 90 % of the oral administrated alcohol enters the metabolism. The rest is unmodified eliminated via urine, sweat and breath [31].

The pathway of alcohol starts with the absorption into epithelial cells, mostly in the small intestine, but also in the stomach and other parts of the gastro-enteric tract. The absorption follows Fick's law of diffusion. The higher the difference in concentration between lumen and cytosol, the more alcohol enters the cell, which means that the dose in which ethanol is consumed modulates the BAC. Drugs and other substances which regulate the stomach motility, may influence the time of absorption and modulate the peak concentration of blood alcohol. A fast stomach emptying leads to higher peak levels [32]. Mechanism and location of the first pass metabolism of alcohol remains a controversial topic, however first pass metabolism seems to be more relevant with low alcohol concentrations (0.3g/kg bodyweight) [33].

1.5.3 Distribution

The volume of distribution of alcohol (V_d) is already mentioned by Widmark as rho factor [27]. The initial rho factor had no dimensions and was calculated as follows:

$$rho = \frac{concentration\ of\ alcohol\ in\ the\ body}{concentration\ of\ alcohol\ in\ blood}$$

Equation 3: Rho factor

Alcohol is highly soluble in water. Men with a high percentage of water have an average $V_d \approx 0.7$ L/kg compared to female with $V_d \approx 0.6$ L/kg [27].

Using this ration the blood alcohol concentration is calculated as follows:

$$BAC = \frac{a}{p\ x\ r}$$

Equation 4: Calculation of blood alcohol concentration according to Widmark [27]
BAC=blood alcohol concentration, a=total dose of administrated alcohol, p=body weight, r=rho (distribution ratio in male= 0.7, in female =0.6)

1.5.4 Elimination

The key enzyme for the oxidative elimination of alcohol is the alcohol dehydrogenase (ADH). We can find the enzyme in cytosol of different tissues, but mainly in the liver. ADH is a dimeric enzyme, consisting of two subunits with about 40 kDa each. The subunits are coded on 7 different genes. According to their enzymatic and genetic characteristics all isoenzymes are divided into 5 different classes. Class I ADH, located in the cytosol of the human liver, has a low Km and the greatest metabolizing capacity [34].

MEOS, a second oxidative pathway for ethanol in the endoplasmatic reticulum of hepatocytes, enhances the degradation of ethanol especially in cases of high BACs. CYP2E1 is a main enzyme in this system and as many cytochrome P450 enzymes inducible by the concentration of substrate. The inter-individual differences in enzyme activity refer primarily to environmental factors, such as pronounced drinking habits [35].

The third oxidative pathway of ethanol elimination takes place in peroxisomes by the catalase. This enzyme occurs also in the brain and seems to play a role in the desire to consume alcohol [36]. There are also non-oxidative pathways, which are according to current data not of quantitative relevance [37]. Zakhari gives a short overview about the ethanol metabolism in general. ADH remains to play the most important role in this procedure.

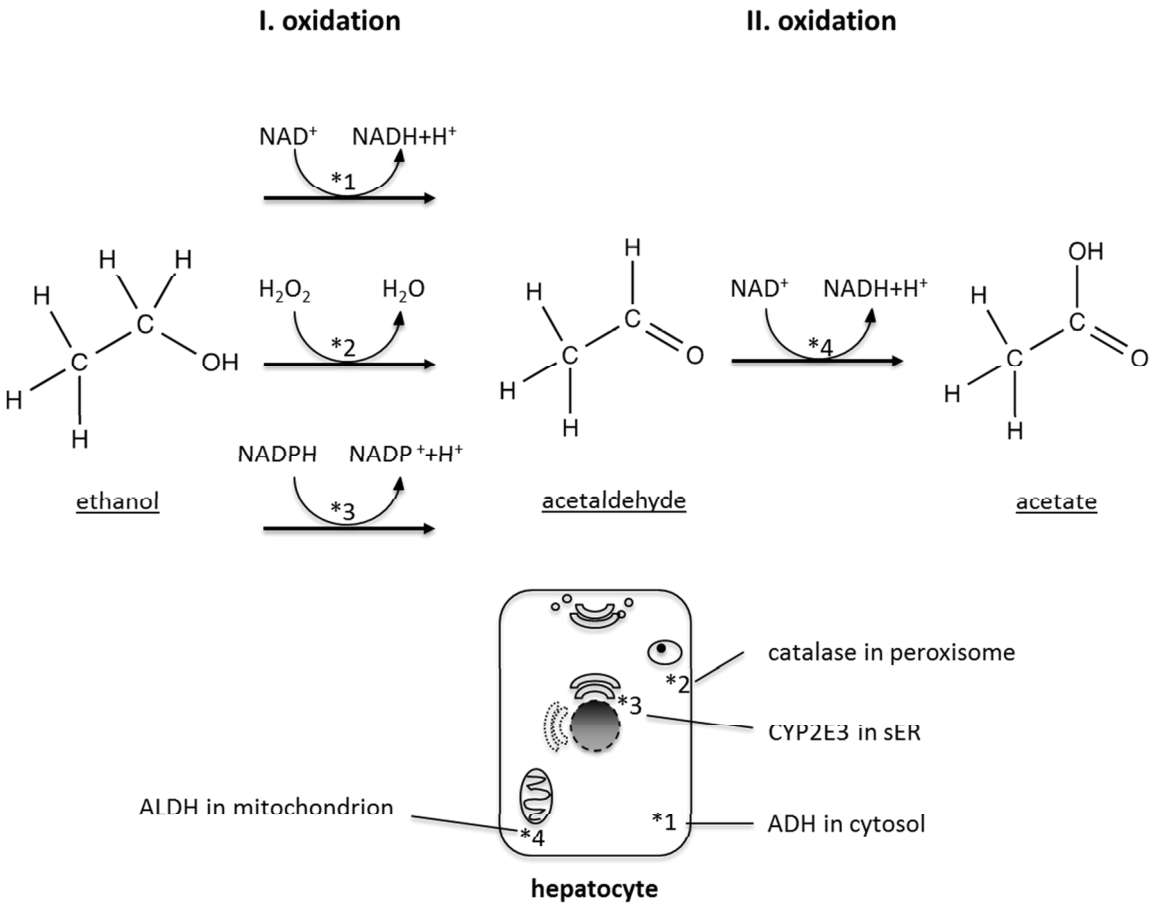

Fig. 2: Oxidation of ethanol following Zakhari [37]

With a low Km of 2-10 mg/100 ml the ADH is saturated at low BAC levels. Concentrations more than 20 mg/100 ml lead to the well-known linear model for 0 order kinetics. Below 20 mg/100 ml, alcohol elimination follows 1st order kinetics [26], [38]. Thanks to the model of 0 order kinetic the elimination rate k can be calculated via linear regression with the following formula:

$$k\ (‰/h) = (Cmax - Cend)/(tend - tmax)$$

Equation 5: Determination of elimination rate k [26]
At time t_{max} (h) the highest concentration C_{max} (‰) is measured, C_{end} is determined as concentration at the last time point (tend) when the measured alcohol concentration is above 0.

The average slope of elimination rate k was first specified by Widmark with β [27]. His results refer to 15.8 mg/100 ml/h in men and 16.8 mg/100 ml/h in women. Comparisons of current results estimate a rate of elimination between 10-15 mg/100 ml/h in fasted and a more rapid one in non-fasted individuals. In conformity with last results of Jones, A. W. [26] females seem to eliminate faster than males. With a lower distribution rate in women,

the overall clearance rate of alcohol seems to be equal in both genders. That means that the time of drunkenness is similar, if the same amount of alcohol per kg bodyweight is given to female and male subjects. Variations in the elimination rate of alcohol occur inter- and intra-individual. This remains a challenge for forensic and clinical research [26].

Following the example of Jones *et al.* [26] of the pharmacokinetic profile of alcohol, a concentration time profile in a placebo treated subject is shown in figure 3. The first part of the graph reflects mainly the absorption phase of ethanol with a rapid increase in concentration. The second part shows a linear decrease and is determined by the elimination of ethanol.

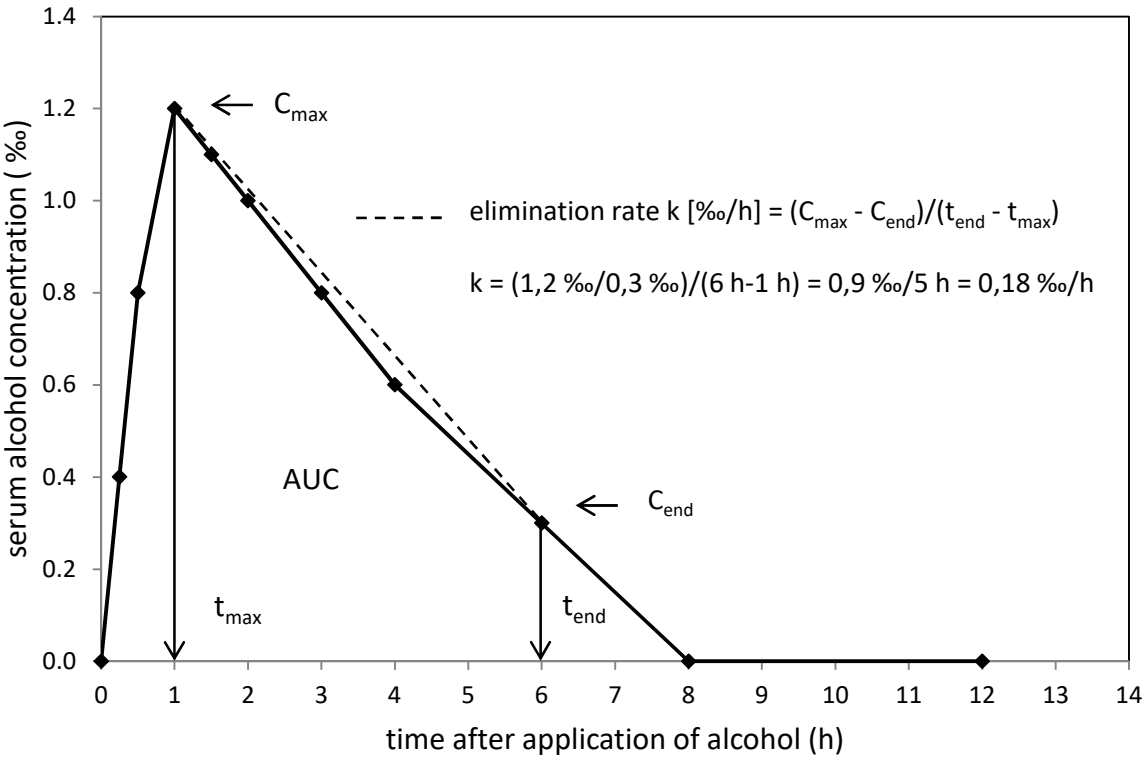

Fig. 3: Pharmacokinetic profile: Subject no. 1 treated with placebo
The sample with the highest highest measured concentration C_{max} (1.2 ‰) was taken at t_{max} (1 h), elimination rate k was calculated as 0.18 mg/g/h

The ethanol concentration in blood measured at 11 time points as we examined it in our study expresses the status of ethanol metabolism in subjects highly sensitive. Clinical trials, which served as model for this research project, are represented in publications of Daldrup and Böhm [39] and Jones *et al.* [26].

2 OBJECTIVES

2.1 General Considerations For Objectives

Following the rationale for conducting the study (see 1.4), the primary aim of this study was to analyse the influence of tiopronin on the metabolism of alcohol in healthy human subjects. Four secondary objectives have been included to obtain further information about the drug's safety and possible consequences of its interactions in the metabolism of alcohol.

2.2 Primary Objective

I. Hypothesis blood alcohol concentration

Tiopronin in healthy subjects (w/m), taken in a therapeutic dose, has no effect on the metabolism of orally administrated ethanol (0.8 g/kg bodyweight), measured in mg/g blood alcohol concentration.

2.3 Secondary Objectives

II. Hypothesis blood acetaldehyde concentration

Tiopronin in healthy subjects (w/m), taken in a therapeutic dose, has no effect on the blood acetaldehyde concentration, measured in µg/ml, before and 2 and 4 hours after an orally administrated ethanol dose of 0.8 g/kg bodyweight.

III. Hypothesis concentration abilities

Tiopronin in healthy subjects (w/m), taken in a therapeutic dose, has no effect on the intra-individual perceptual speed, measured by the number connection test of Oswald & Roth, two hours after an orally administrated ethanol dose of 0.8 g/kg bodyweight .

IV. Hypothesis breath alcohol concentration

Tiopronin in healthy subjects (w/m), taken in a therapeutic dose, has no effect on the breath ethanol concentration, measured in mg/l, one hour after an orally administrated ethanol dose of 0.8 g/kg bodyweight.
Tiopronin in healthy subjects (w/m) has no effect on the breath ethanol concentration compared to blood alcohol concentration.

V. Hypothesis safety evaluation

Tiopronin in healthy subjects (w/m), taken in a therapeutic dose, has no effect on the safety of healthy subjects after an orally administrated ethanol dose of 0.8 g/kg bodyweight.

3 METHODS

This was an investigator-driven randomised, double blind, cross-over study about the interaction of tiopronin on the metabolism of alcohol and the medications safety in this combination in healthy volunteers. Idea, support and supervision came from my supervisor Dr. Dr. med. Michael Kroll (see 12.) who acted as principal clinical investigator (Leiter klinische Prüfung, LKP). The author of this thesis was the main investigator. Sponsor of the capsules with tiopronin and with placebo was MIT Gesundheit GmbH, the producer of Captimer on the international market. The sponsor did not have any influence on the actual conduct of the study, on the analysis of the results and the content of the publication (see 12.).

The study was conducted in accordance with the “Note for Guidance on Good Clinical Practice” (CPMP/ICH/135/95), performed according to the Revised Declaration of Helsinki (2013), and was officially approved as AMG study by the Ethics Committee of the Regional Medical Association (Nordrhein) (Zeichen 2014060, dated 20.5.2014) [1]. The letter of approval is available in appendix 10.1.

3.1 LITERATURE RESEARCH

3.1.1 General considerations

The literature research took place between April 2013 and March 2016 and consisted of three main fields: 1.) tiopronin, 2.) cystinuria and 3.) alcohol metabolism, and the three fields' combinations. I chose Medline via PubMed as main database, but received additional information from the sponsor (MIT Gesundheit GmbH) which was partly not publicly available but essential for this work. The following abstract summarises the literature research process. All articles chosen for analysis and named are referenced in chapter 7 (References).

3.1.2 Tiopronin

2013, April:

Before the literature research started I received two main articles from my supervisor Dr. Dr. Kroll. The first article of Hoshino *et al.* (1985) is the only available source where the influence of tiopronin on the metabolism of living organisms is investigated. Moreover I received a paper of Ogata *et al.* (1968) about the first use of tiopronin in the treatment of the Minamata disease in Japan.

2013, Mai:

The aim of the first research round in April and Mai 2013 was to gain general information about tiopronin and to probably extract studies with the same research question.

PubMed showed 922 results using the key words "tiopronin OR alpha-mercaptopropionylglycine OR 2-N-mercaptopropionylglycine". 32 results were reviews and additionally in English, German or French language. A review of Knoll *et al.* (2004) gave a good overview about tiopronin and its main indication cystinuria. The article referred to Chow *et al.* (1997) as clinical trial to evaluate the best treatment for cystinuria and to Harbar *et al.* (1986), who compared D-penicillamine and tiopronin. Latter was not used for this thesis but for the article in "drug research"[1].

Primary objective (see 2.2): The combination of "tiopronin OR alpha-mercaptopropionylglycine OR 2-N-mercaptopropionylglycine AND alcohol" resulted in 50 findings. Most of the results refer to tiopronin as a reducing agent, radical scavenger or

thiol compound in fundamental or preclinical research. In this context Tiopronin is used as part of a method but not as main interest. A few animal studies on mices or rats analysed tiopronin as a protective drug after metal or ethanol intoxication. No study examined the effect of tiopronin on the blood alcohol concentration. Adding clinical trial as article type there were 0 results, giving the first impression that the objectives of this study had not been investigated in a clinical trial so far.

The key words "tiopronin OR alpha-mercaptopropionylglycine OR 2-N-mercaptopropionylglycine" chosen for the article type 'clinical trial' showed 38 results in PubMed, 26 of it in Englisch, French and German language. I extracted the articles form different research fields: Amor *et al.* (1982) about the antirheumatic treatment with tiopronin in France, Kim *et al.* (2010) a phase I clinical trial about the neuroprotective function of tiopronin and Chow *et al.* (1996). Some of the other articles also referred to tiopronin's characteristic as radical scavenger.

In the next step different key words mentioned in the 26 clinical trials such as radical scavenger, neuroprotection, liver or heart protection were re-evaluated.

"Tiopronin OR alpha-mercaptopropionylglycine OR N-2-mercaptopropionylglycine OR N-2-MPG AND neuroprotection" in French, English or German language showed 5 results. Besides Kim *et al.* (2010), Ivanova *et al.* (2002) was selected.

"Tiopronin OR alpha-mercaptopropionylglycine OR N-2-mercaptopropionylglycine OR N-2-MPG AND rheumatoid arthritis" in French, English or German language showed 72 results. 8 of them were clinical trials. Amor *et al.* (1982) remained the most interesting for this thesis.

"Tiopronin OR alpha-mercaptopropionylglycine OR N-2--mercaptopropionylglycine OR N-2-MPG AND radical scavenger" in French, English or German language showed 52 results. None of them were a clinical trial. After a reselection Ortolani *et al.* (1982) seemed to be an early but still relevant source.

"Tiopronin OR alpha-mercaptopropionylglycine OR N-2-mercaptopropionylglycine OR N-2-MPG AND heart protection" in French, English or German language showed 28 results. Finally I chose none of them.

"Tiopronin OR alpha-mercaptopropionylglycine OR N-2-mercaptopropionylglycine OR N-2-MPG AND liver protection" in French, English or German language showed 5 results. Finally I chose none of them.

To conclude information about tiopronin I looked for “tiopronin[Title] OR alpha-mercaptopropionylglycine[Title] OR N-2--mercaptopropionylglycine[Title] OR Captimer[Title] OR thiola[Title]”. There were 197 results, 150 in English, German or French language. 7 sources were selected and included in the work namely: Kim *et al.* (2010), Fetoni *et al.* (2004), Fujimoto *et al.* (1979), Amor *et al.* (1982), Ortolani *et al.* (1982), Remien *et al.* (1975) and Devi *et al.* (1983).

3.1.3 Cystinuria

Cystinuria is the main indication for tiopronin according to the current SPC of tiopronin [4]. This study was conducted to gain information for patients and physicians; therefore a literature research giving general information about the patient group was examined. Main parts of this literature were provided by the sponsor and are listed below.

2013, April:

In the first round in April 2013 the research was focused on reviews about cystinuria. Under the heading “cystinuria” in PubMed 1.586 results were found. 192 of them were reviews in English, German or French. Cystinuria appeared in 43 results in the title. Of these 43 I extracted Eggermann *et al.* (2012) with a general overview about the disease, Knoll *et al.* (2004) and Crawhall *et al.* (1968) as an early but detailed and systematic review. Relevant references of the articles were Dello Strologo *et al.* (2002) with a focus on different genetic and phenotypic classification of cystinuria. The article of Weinberger *et al.* (1974) gives information about the incidence in Jewish populations, and Crawhall *et al.* (1963) firstly treated cystinuric patients with D-penicillamine.

2013, October:

In the second round in October 2013, I re-evaluated the first findings and looked for key words “cystinuria OR cystine stones OR cystine calculi”. PubMed found 2.204 results. 1920 article were listed in German, French or English. 279 remained as reviews. 64 of the review were published between the years 2005 and 2013. Ahmed *et al.* (2006) was selected.

Meanwhile I received a chronologically ordered literature file about cystinuria and tiopronin from the sponsor. The following literature was adopted from this file: SPC

tiopronin (2008), SPC penicillamine (2013), Biyani *et al.* (2006), Wollaston (1819), Berzelius (1779-1848), Tabachnick *et al.* (1954) and Crawhall *et al.* (1963).

3.1.4 Alcohol metabolism

2014, January

Before the literature research for the field of alcohol metabolism started in January 2014, I gratefully received the original source of Widmark (1932) from my supervisor Dr. Dr. Kroll.

2014, February

For the key words “ethanol OR alcohol” PubMed found 780.474 results. For the keywords “elimination of alcohol OR elimination of ethanol” PubMed found in German, French or English language 5.716 results. 410 results remained when only reviews were included. 36 reviews remained when “elimination of alcohol OR elimination of ethanol” had to be part of the title. Three articles were selected: Jones *et al.* (2010) concluded a big knowledge about the metabolism of alcohol and gave a good introduction into the field of clinical studies. It remained a main paper until the end of this research project. Moreover Holford *et al.* (1987) and Crabb (1997) were acquired. These three sources refer to following chosen literature: Kalant *et al.* (1971), Lieber *et al.* (2004), Zakhari *et al.* (2006), Edenberg *et al.* (2007), Jones *et al.* (2003) and Wagner *et al.* (1989).

2014, March:

My supervisor Dr. Dr. Kroll found sources about the influence of tiopronin on the blood levels of acetaldehyde. After reading different sources, Zimatkin and Deitrich (1997), Font *et al.* (2005) and Font *et al.* (2006) were adapted for this thesis.

2016, January:

Looking for more currently available data in all three research fields 1.) tiopronin, 2.) cystinuria, 3.) alcohol metabolism, a study from Hartung *et al.* (2016) with current results of modern breath alcohol measurements was found under the keywords “breath alcohol measurements OR breath ethanol measurements” and included.

2016, March:

With the heading “alcohol[Title] OR ethanol[Title] AND (medication[Title] OR drugs[Title]) AND interaction[Title]” PubMed showed 38 results of which Weathermon

and Crabb (1999) was chosen. This article summarises many drug alcohol interactions, but does not mention tiopronin.

Other databases and sources:

Three sources about alcohol metabolism and measurements were primarily received from 'google'. Looking with the heading "blood alcohol concentration international units" google found 682.000 results in June 2014. One of the first results was a short article on encyclopedia.com from Jones A.W. (2001). This article gives a good overview about international units and refers to Jones *et al.* (1989). Aderjan *et al.* (2011), a current guideline for alcohol measurements in forensic case work, was found as first result out of 8.850 with the heading: "Blutalkohol AND Richtlinien" in January 2015. An article about the influence of ceftriaxone on the metabolism of alcohol from Daldrup and Böhm (1982) was found during the preparation of acetaldehyde measurements in cooperation with the Institute for Legal Medicine in Düsseldorf in June 2015.

Information about the applied device to measure blood alcohol (cobas c system; see 3.4.2.1) was provided by Annette Abraham from the central laboratory Goch (hospital). Dräger Saftey provided not only the breath measurement device (see 3.4.2.4) but also an accompanying booklet. Information about the ZVT of Oswald and Roth (1987) (see 3.4.2.3) are part of the purchased test manual. This manual refers to Reitan *et al.* (1956).

3.2 SUBJECTS OF THE STUDY

With this sample size of 12 a significant result of the paired t-test on a two-side 5% level is expected with power 90%, if the difference between the expected values μT and μP of log(AUC) is the same amount as their standard deviation [1]. Subjects were recruited from the University of Düsseldorf and from Rhine-Waal University. A recruitment poster is attached in appendix 10.2.1. After having met all inclusion and no exclusion criteria, seven male and seven female subjects between 18 and 30 years were successfully included into this trial. Due to personal reasons one participant dropped out after inclusion but before receiving any study medication, so that 13 individuals passed the clinical trial. All subjects were stated healthy according to physical examination, blood-, urine- and ECG- analysis. Apart from hormonal contraceptives in females, none of them were allowed to take any additional medication. Pregnancy was reliably excluded in every female

individual. All subjects gave informed consent [1]. The subject information and an example file to give informed consent are attached in appendix 10.2. To document all examinations, treatments, adverse events and safety measurements a Case Report Form was created (appendix 10.3).

All inclusion and exclusion criteria are shown in the following.

3.2.1 Inclusion criteria

The following inclusion criteria in table 2 had to be met by every subject:

Inclusion criteria
a) age 18 - 40 years (inclusive)
b) "healthy" as judged after a medical and laboratory examination by a physician
c) either male or female
d) female subjects: reliably excluded pregnancy
e) signed informed consent

Table 2: Inclusion criteria

3.2.2 Exclusion criteria

None of the exclusion criteria of table 3 were allowed to be met by any subject:

Exclusion criteria
a) unable or unwilling to abide reliably by the requirements of the study protocol
b) female subjects who neither are using contraceptives nor being postmenopausal nor being surgically sterile, thus being of childbearing potential, but not exhibiting a negative pregnancy test
c) females who are pregnant or breast feeding
d) hemodynamically relevant atrial or ventricular arrhythmia (for example subjects with uncontrolled atrial fibrillation or a ventricular response rate greater than 100 or frequent [> 10/min] premature ventricular complexes at rest)

e) evidence of cerebrovascular accident within the last 6 months
f) clinically significant pulmonary, hepatic, gastrointestinal, neurologic or hematological disease
g) anamnestic suspected impairment of renal function, acute or chronic liver diseases, heart disease
h) surgery or disease of the gastrointestinal tract or of other organs which in the opinion of the investigator might influence resorption or elimination of the drug
i) acute autoimmune disease or clinically significant acute endocrine disease
j) active wasting disease including cancer
k) psychological and/or emotional problems which would render the informed consent invalid
l) current or anamnestic drug addiction, or extensive chronic use of alcohol, or history of alcoholism (so called "dry alcoholics")
m) any co-medication (except oral anti-contraceptives in women)
n) participation in another study with another investigational drug within 6 weeks prior to study enrolment
o) limitation in exercise capacity due to other reasons than heart failure (e.g. physical impairment)

Table 3: Exclusion criteria

3.3 STUDY DESIGN

3.3.1 Overall study design

This was a randomised, double-blind, cross-over, phase I study in healthy volunteers. Each subject served as his own control. The nature of the study and the potential risks associated with the trial drug were explained to all candidates. After written informed consent and checking, if inclusion criteria were met and exclusion criteria were not met, suitable subjects were included into the study.

After overnight fasting, subjects appeared approximately at 6:00 am in the morning. Each volunteer received in one period at about 7:00 am 500 mg tiopronin (2 x 250 mg tablets in one capsule) and in the other period one capsule of identically looking placebo. The sequence tiopronin-placebo (Sequence A-B) or placebo-tiopronin (Sequence B-A) for all volunteers was prospectively randomised and double blinded; both periods were separated by a 1 week wash-out period (11/2015) [1].

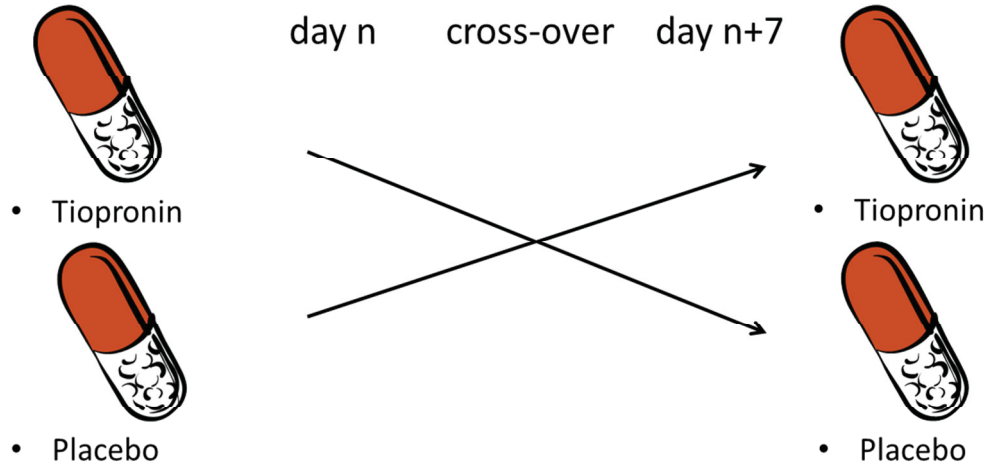

Fig. 4: Cross-over study design

The randomisation took place according to the pattern of a latin square in blocks of four as shown in table 4. There were two different symbols defined as A and B. A symbolises the placebo treatment and B the treatment with tiopronin in the first period. Within one block of four boxes, each symbol has to appear two times. A cube determines the first and second symbol of the box. If two identical symbols were rolled (as shown in the second block), the two empty boxes obtain the other symbol. If two different symbols were rolled (as shown in the first block), the dice has to determine the symbol of the third box. The forth symbol is always determined by its predecessors.

Example:

A
B
B
A
A
A
B
B

Table 4: Latin square in blocks
"A" symbolises the placebo treatment and "B" the treatment with tiopronin in the first period. Within one block of four boxes, each symbol has to appear two times. A cube determines the first and second symbol of the box. The forth symbol is always determined by its predecessors.

Each subject received an envelope with the date of the study day, the name and the subject number on its cover. The envelope contained furthermore the information of the subject's treatment on this study day. This information was written on a separate paper and not apparent from the outside. "A" meant placebo and "B" tiopronin treatment in the first study period.

Neither of the study group members was allowed to be informed about the randomisation list, which was created by the head of the pharmacovigilance department of MIT Gesundheit GmbH. After the data recruitment had been closed (05.02.2016) the regular un-blinding took place (21.02.2016). Cases which required a premature unblinding, for instance severe adverse events, were defined in the study protocol, but did not occur.

One hour after application of the test drug all volunteers received 0.8 g/kg body weight pure alcohol in form of 40 % vodka. Blood samples were collected at the following time points: 0 (after application of test drug but before application of alcohol) and 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h after application of alcohol. The alcohol concentration was measured in samples from each time point. All profiles followed the expected 0 order kinetic and were close to the estimated alcohol concentration of 1.1 ‰ or 1.3 ‰. The acetaldehyde concentration was measured in blood samples at the time points 0 h, 2 h and 4 h after application of alcohol. To obtain information about concentration abilities, which could be affected by alcohol consumption the ZVT (a number-connection test) was performed before and 2 h after alcohol intake. Additionally, safety parameters such as RR, pulse and oxygen saturation as well as breath alcohol concentration were collected.
According to the Widmark formula males should have an estimated blood alcohol concentration of 1.14 ‰ and females of 1.33 ‰ [1].

$$BAC = \frac{a}{p \; x \; r}$$

Equation 6: Calculation of blood alcohol concentration according to Widmark [26]
BAC=blood alcohol concentration, a=total dose of administrated alcohol, p=body weight, r=rho (distribution ratio in male= 0.7, in female =0.6)

The schedule of one study day is shown in the following flow charts (Fig. 5):

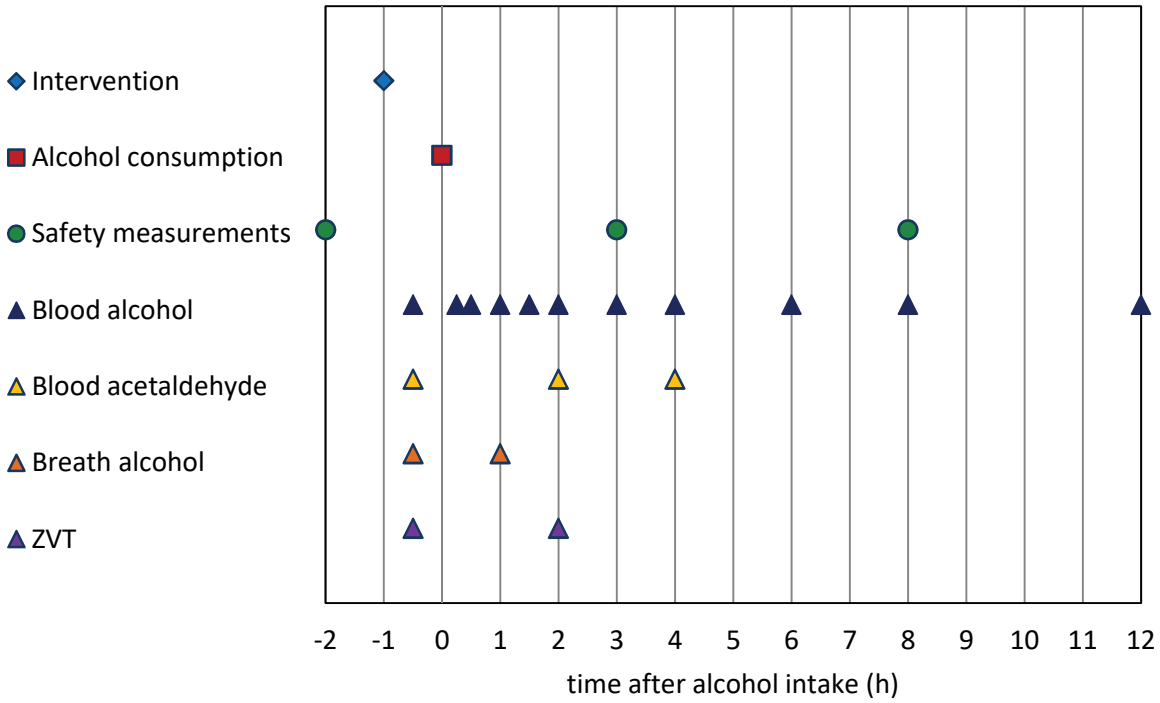

Fig. 5: Schedule of one study day [1]

3.3.2 Duration of the study

Pre-screening procedure and inclusion of 14 subjects were performed between the 05.10.2015 and 28.10.2015. Due to personal reasons one participant dropped out after inclusion but before receiving any study medication (see 3.2).

The first study day was the 14.11.2015 and the second study day was the 21.11.2015. Data on the primary and secondary objectives was collected on these dates.

Within 4 weeks after the last study day each of the 13 participating subjects was examined in a post study visit by the principle investigator. Two subjects needed a second after study appointment with the principal investigator and a second blood check to re-evaluate increased blood values of leucocytes. In both cases normal blood values could be found in the second examination (21.01.2016 and 03.02.2016).

Adverse event data collection was closed on 03.02.2016. A close-out monitoring report was available on 05.04.2016. The first full study report draft was available on 09.04.2016.

An overview of the study schedule and the procedures to be completed at each visit is given in the CRF (see appendix 10.3). Additionally safety parameters collected on the

study days such as blood pressure, heart rate and O_2 % were hand written and signed by the principle investigator.

The timeline of the involved months is shown in the following flow chart (Fig 6).

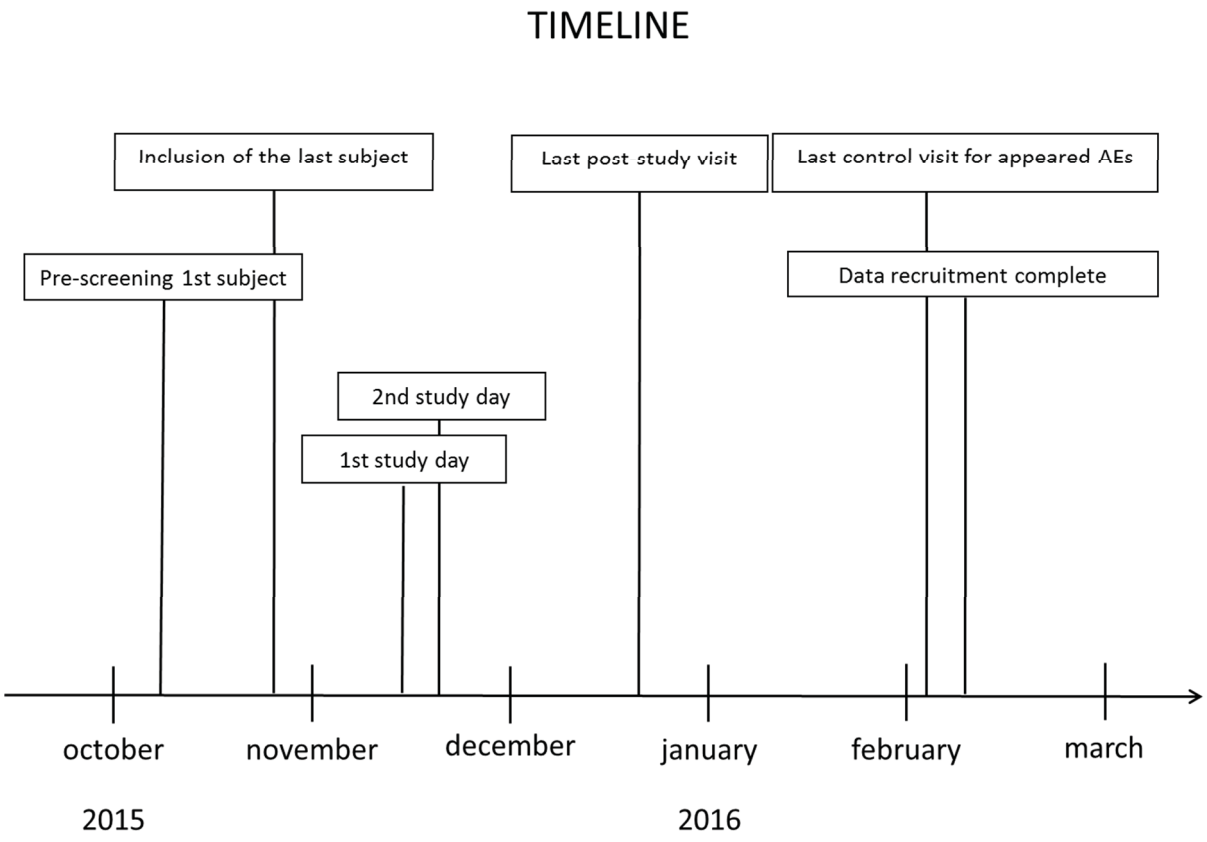

Fig. 6: Duration of study
Pre-screening of the first subject: 05.10.2015; inclusion of the last subject: 28.10.2015; 1st study day: 14.11.2015; 2nd study day: 21.11.2015; last post-study visit: 22.12.2015, last control visit for appeared AE's: 03.02.2016; data collection complete: 05.02.2016

3.3.3 Organisation of the study team

To assure the quality of sample and data collecting, all study assistants were trained during the two weeks, before the first data was collected. Tasks were organised into 5 task areas.

Task area 1 named "Vigo & Blut" included:

a) preparation of surgery room and necessary material for venous accesses and disposable venous puncture, including non-alcoholic dabs

b) verification of subjects' identity and CRF

c) sampling of blood with note of time and signature

d) cooling of samples

e) centrifuging and pipetting of samples

f) delivery to sample carrier

Task area 2 named “Dräger Alcotest 6810” included:

a) preparation of Dräger Alcotest 6810

b) verification of subjects’ identity

c) performance of Dräger Alcotest 6810 according to the instruction manual

d) collecting of breath alcohol samples

e) note of value, time and signature

Task area 3 named “ZVT” included:

a) preparation of ZVT files

b) performance of the psychometric test according to the instruction manual

c) note of time and signature in each subjects CRF

d) note and evaluation of test results

Task area 4 named “Zeitplanung/Einnahme Substanzen” included:

a) identification and labelling of each subject in the morning of each study period

b) supervision of each subject during the whole study period

c) supervision of the drug intake according to protocol

d) supervision of the alcohol intake according to protocol

e) note of time and signature of both intakes

f) assurance of time schedule

g) assurance of subjects well-being

Task area 5 named “Untersuchung” included:

a) examination of subjects’ health status in the morning of every study period

b) examination of safety parameters as stated in the study protocol

c) examination of safety parameters whenever necessary or required by Dr. Pelzer (emergency physician)

d) note of surveyed safety parameters, time and signature

e) note and treatment of any adverse event (AE)

f) immediate forwarding of AEs to the monitor

A time schedule including all task areas was distributed and discussed with all study team members to ensure the trials' quality. A sample of a time schedule without subject information, a building plan and information about the study team are available in appendix 10.4.

3.4 MEASUREMENTS

3.4.1 Safety measurements

3.4.1.1 General Safety Measurements

General safety of assessments was given through the strict compliance with procedures as documented in the CRF. The CRF includes information about the parameters which were controlled systematically during the two study days.

According to CRF during the study days the following vital parameters were monitored at 6:00 am, at 10:00 am and at 14:00 am. The latter two values were recorded handwritten.

a) blood pressure (Korotkow),

b) pulse rate,

c) arterial oxygen saturation, based on the photoelectric plethysmography

Moreover, the emergency physician Dr. Pelzer was available during the whole period.

3.4.1.2 Physical examination

As part of the pre-screening process it was important to carefully check the subjects' symptoms and signs. The physical examination performed by the principal investigator comprised measurements of the following parameters (table 5):

Measurable parameters	Results of acceptance
Blood pressure	Systolic: 100 - 145 mmHg Diastolic: 55 - 90 mmHg Arterial blood pressure measurement was carried out by using a standard calibrated sphygmomanometer and a properly sized cuff or any other calibrated device. When using a sphygmomanometer, sitting systolic pressure was recorded when the initial sound was heard (Phase 1 of the Korotkoff sound), diastolic pressure was recorded at the disappearance of the sound (Phase V of the Korotkoff sound) with the arm supported at the level of the heart.
Pulse	45 - 85/min, rhythmic
ECG	Without diagnostic findings
Temperature	36 °C – 37.5 °C
Skin, Shape, Palpation lymphatic knots	Normal complexion, normal vascular drawing, symmetrical build, without oedema, small (< 1 cm, apart from inguinal l.k.) or not palpable, relocatable lymphatic knots without tenderness on palpation
Auscultation thorax	clear heart sounds, no cardiac murmurs vesicular pulmonic breath sound, no side noise
Palpation abdomen	Without tenderness on palpation (abdomen and renal bed), resistance, muscular defence, ascites regular sized liver and spleen
Neurologic tests	Pupillary reflex Triceps reflex Knee-jerk reflex Babinski-sign negative
BMI	Between 19 - 28 (inclusive)

Table 5: Reference values in clinical examination

3.4.1.3 Laboratory assessment

Assessments of laboratory parameters were performed in an accredited laboratory. Urinalysis (dip-stick) as well as urine pregnancy test in females were performed onsite. The following laboratory analysis was performed (see table 6):

Measurable parameters	Results of acceptance		
Blood diagnostic	Early morning, fasting subject, EDTA, Serum, Citrated blood		
Pregnancy test (mandatory in women)	Negative		
Small blood count	Haemoglobin	male: 13 - 17 g/dl	female: 12 - 16 g/dl
	Haematocrit	male: 42 – 50 %	female: 38 – 44 %
	Erythrocytes	male: 4.3 – 5.6 mill/µl	female: 4.0 – 5.4 mill/µl
	MCV MCH MCHC	85 – 98 fl 28 – 34 pg 31 – 37 g/dl	
	Thrombocytes	140 - 345 x 1000/µl	
	Leukocytes	3800 - 9500 /µl	
Inflammation marker	CRP	< 5 mg/l	
	BSG	< 20 mm/h	
Coagulation laboratory	Quick	≥ 70%	
	INR	~1	
Organ specific parameter	γGT	male: < 60 U/l	female: < 40 U/l
	GOT	male: ≤ 38 U/l	female: ≤ 34 U/l
	GPT	male: ≤ 41 U/l	female: ≤ 31 U/l
	Bilirubin total	≤ 1.1 mg/dl	
	CK	Male: < 190 U/l	female: <170 U/l
	Creatinine	< 1.1 mg/dl	
	Urea	12 - 50 mg/dl	
Metabolic parameters	Uric acid	< 7 mg/dl	
	Glucose	≤ 100 mg/dl (fasting) < 130 mg/dl (after breakfast)	
Proteins	Proteins total	66 - 83 g/l	
Electrolytes	Sodium	135 - 145 mmol/l	
	Potassium	3.6 – 5.0 mmol/l	
	Calcium total	2.2 – 2.6 mmol/l	
Urine	10 ml central ray, early morning urine		
	Pregnancy test (females only)	Negative	
	pH-value	5 – 8	
	Proteins total	< 15 mg/dl	
	Glucose	Negative	
	Acetone	Negative	
	Blood	Negative	
	Nitrite	Negative	
	Bilirubin	Negative	
	Urobilinogen	Negative	
	Leucocytes	Negative	

Table 6: Reference values in laboratory assesments

Interpretations of the laboratory results were performed by the same physician who performed the physical examination.

3.4.1.4 Recording of adverse events

All adverse events (AEs), which were reported by subjects or observed by the investigator, were recorded on the appropriate CRF page ("AE") regardless of their causal relationship (see table 7). There was no serious adverse event (SAE) as defined from the U.S. Food and Drug Administration during the clinical trial [40].

	Adverse Event (per line only one single event!)	Seri -ous	Start End	Intensity	Relationship to study drug	Actions taken	Outcome
1		☐ yes ☐ no	␣␣/␣␣/␣␣ dd / mm / yy ␣␣/␣␣/␣␣ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ unknown ☐ not related	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
2		☐ yes ☐ no	␣␣/␣␣/␣␣ dd / mm / yy ␣␣/␣␣/␣␣ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
3		☐ yes ☐ no	␣␣/␣␣/␣␣ dd / mm / yy ␣␣/␣␣/␣␣ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
4		☐ yes ☐ no	␣␣/␣␣/␣␣ dd / mm / yy ␣␣/␣␣/␣␣ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown

Table 7: Documentation of adverse events

3.4.2 Efficacy measurements

3.4.2.1 Blood Alcohol Concentration

The metabolism of alcohol as it is explained in chapter 1 is the underlying base for all measurements.

For the analysis of alcohol, blood was drawn at the following time points: 0 (after application of the test drug but before application of alcohol) and then 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h after application of alcohol. The blood samples which were analysed by the central laboratory Goch contained separation gel.

Mechanism and preparation of the applied cobas c system (Roche/Hitachi) [41] are explained in the following lines:

The cobas c system of Roche /Hitachi is a quantitative, enzymatic in-vitro test, evaluating the alcohol concentration of human plasma, serum and urine. The mechanism is based on a photometric measurement of NADH, which results from the redox reaction between ethanol and NAD+, catalysed by alcohol dehydrogenase (ADH). The reaction is identic to the predominant hepatic metabolism of alcohol in vivo.

$$Ethanol + NAD(+) \; --ADH--\rightarrow Acetaldehyde + NADH(+) + H(+)$$

Changes in photometric absorption based on the increasing $NADH^{+}$ concentration, with a specific light absorption at 340 nm, are directly proportional to the ethanol concentration.

The blood sampling was carried out with non-alcoholic disinfectants and suitable tubes *VACUETTE® TUBE 9 ml Z Serum Separator Clot*. Unlike the tubes used for acetaldehyde concentrations, these tubes contained a separation clot, to facilitate further procedures.

The durability of the samples depends on the surrounding temperature:

15 - 25 °C	2 days
2 – 8 °C	2 weeks
(-15) – (-25) °C	4 weeks

The samples were completely closed and presented at the laboratory the same day. The sample volume was set at 4 µl. Using the commercial test system cobas® (cobas c system of Roche /Hitachi) (see above), two reagents were added. Reagent 1 (50 µl) contained buffer and preservatives, reagent 2 (50 µl) contained NAD+ (yeast) ≥3.3mol/l, ADH (yeast, 25°C) ≥617 µkat/l, buffer, stabilisers and preservatives. The linear calibration was determined between concentrations of 13.1-447 mg/dl. Results of measurements were reported in ‰ (g/kg) in serum.

The enzyme-based analytic measurement of ethanol has a measurement range between 10.1 and 498 mg/dl. Precision is indicated with < 5 % V (coefficient of variation), the correlation coefficient with other methods is > 0.982 [1].

3.4.2.2 Blood Acetaldehyde Concentration

Blood for further acetaldehyde analytics in the forensic laboratory Düsseldorf was sampled in VACUETTE® TUBE 9 ml Z Serum Clot Activator without further additives. These samples were taken at following time points: 0 h, 2 h and 4 h after application of alcohol. Furthermore, they were centrifuged and serum was pipetted at least 2 hours after collection. After storage for 48 h at 4 °C they were transported to the laboratory, without interruption of the cold chain.

Using headspace gas chromatography, the forensic laboratory in Düsseldorf examined a new method to determine acetaldehyde concentrations noted in µg/ml. The authorship and copyrights of the method, described in the following, belongs to their respective owner B. Kardel:

The headspace gaschromatography were processed by devices of Perkin-Elmer (GC: „Clarus 500“ with FID, HS-Sampler: „HS 110“). The sample volume was set at 200 µl serum and contained tert.- butanol in water as internal standard. The linear calibration (R^2=0,9991) was determined between concentrations of 0,1 – 10 mg/L acetaldehyde.

Serum and internal standard were given into a HS-vial. The vials were tightly sealed with a teflon septum, before heated. Reaching an equilibrium between concentration of sample in liquid and gaseous phase, parts of the gaseous phase in the headspace were injected into a capillary separation column. Volatile comparts were separated and detected with a flame ionisation detector (FID). For the quantification the quotients of signal intensity of acetaldehyde and internal standard are calculated and checked against a calibration curve with 8 levels [1].

3.4.2.3 Number-connection-test (ZVT)

This paper pencil test is a timesaving and economic possibility to evaluate the cognitive abilities of the subjects before and after taking tiopronin/placebo and alcohol. Information processing speed was assessed by the Number-Connection-Test of Oswald and Roth, 1987 [42] which is a variant of the Trail Making Test of Reitan, 1958 [43]. The NCT is a specific language free psychometric intelligence test, which measures primary the speed of

performance known as perceptual speed. The second revised edition of W. D. Oswald, E. Roth, (1987) was utilized. This test comprised four matrices (A, B, C, D) of 90 numbers each arranged in an arbitrary sequence. Participants were required to connect the numbers as quickly as possible in the correct sequence by drawing a line between the numbers using a pencil. The Investigator arranged standardized paper sheets with numbers between 1 and 90, which are randomly distributed. There were four different matrixes, which all had to be fulfilled one after another. The investigator measured the time in all four cases and calculated the average. The evaluation is made out of standard tables, which include T-standard, C- and IQ- values. The average time to complete each matrix was used as performance index. Converted in bit/sec the values were used for intra-individual comparisons between the two study days [1]. An example file of one matrix is attached in appendix 10.5.

3.4.2.4 Breath alcohol concentration

This study used Dräger Alcotest 6810 as a fast measurement for breath ethanol concentrations noted in mg/l (ethanol mass per breath volume at 34 °C and 1013 hPa). Dräger Alcotest 6810 works via an electrochemical sensor in a measurement range between 0.00 mg/l to 2.50 mg/l. The samples were collected at the end of the breathing maneuver. A breath volume > 0.3 L and a breath flow of > 3 L/min were necessary to obtain data. All conditions demanded in the operating manuals of Dräger Alcotest 6810 were fulfilled [44].

3.4.3 Measurement of treatment compliance

In this study a single drug application of verum and placebo given under direct supervision of the investigator was used. Therefore no special measurements for compliance of the drug intake were necessary. The same consideration concerning compliance was valid for the drinking of alcohol which was also performed under supervision of the investigator. A fasting status was assumed but not controlled via blood glucose levels or similar methods.

3.5 STATISTICAL ANALYSIS

The statistical analysis was planned and examined together with Prof. em. Dr. Berthold Schneider.

The AUC is the primary efficacy criterion. The AUC-values calculated for each volunteer at both periods are assumed as realisations of independent normally distributed bivariate random vectors with mean μ1 resp. μ2 and identical variances and covariance for each volunteer. Analysis is based on a mixed linear model with fixed treatment-effect α (difference between the treatments averaged over the periods), fixed period effect β (difference between periods averaged over the treatments), fixed interaction term γ (difference of the treatment effects between both periods) and a random subject effect z. The null-hypotheses (no effects) are tested with F-test (resp. t-test). If the interaction effect can be assumed as 0, the treatment effect can be tested also with paired t-test. The significance level for a two-sided test is fixed to 5 % (one-sided to 2.5 %).

Similar analyses are used for the secondary efficacy criteria (C_{max}, t_{max}, psycho-test). The safety parameters are analysed with descriptive statistics (mean, standard deviation, frequency).

Estimates of the kinetic parameters t_{max}, C_{max}, AUC and k are calculated from the observed concentration curves of each patient at both periods. The data derived from the concentrations of a volunteer are considered as independent and identical distributed realizations of bivariate, normally distributed random vectors with expected values μ_{ij} (where the first index i stands for the sequence (tiopronin-placebo:=1, placebo-tiopronin:=2) and the second index j for the period (1 or 2)), variances σ_j^2 (j=1,2) and covariance σ_{12}. The variances and covariance are assumed as not depending on the sequence i.

As new parameters the grand mean μ, treatment effect α, period effect β and interaction effect γ are defined as orthogonal linear contrasts of the means μ_{ij}:

$$\mu = \frac{1}{4}((\mu_{11} + \mu_{12}) + (\mu_{21} + \mu_{22})) \quad \alpha = \frac{1}{4}((\mu_{11} - \mu_{12}) - (\mu_{21} - \mu_{22}))$$

$$\beta = \frac{1}{4}((\mu_{11} - \mu_{12}) + (\mu_{21} - \mu_{22})) \quad \gamma = \frac{1}{4}((\mu_{11} + \mu_{12}) - (\mu_{21} + \mu_{22}))$$

These new parameters decompose the means μ_{ij} in:

$$\mu_{11} = \mu + \alpha + \beta + \gamma \qquad \mu_{12} = \mu - \alpha - \beta + \gamma$$

$$\mu_{21} = \mu - \alpha + \beta - \gamma \qquad \mu_{22} = \mu + \alpha - \beta - \gamma$$

The grand mean μ is the arithmetic mean of the 4 figures μ_{ij}, and indicates the general location of the data. The effects α and β indicate deviations from the grand mean: α indicates the effect of tiopronin, i.e. the deviation of the means with tiopronin from μ. The effect of placebo is indicated by –α. β indicates the effect of the first period (deviation of the means in the first period from μ) and –β that of the second one. The interaction γ indicates the difference of the tiopronin effect in the first period from that in the second one.

Estimates of the grand mean and effects by replacing in the formulas above the expected values μ_{ij} with the corresponding sample means $\bar{y}_{ij}$. From the formulas it is seen that these estimates are proportional to sums or differences between sequences of sums or differences of the sample means $\bar{y}_{ij}$between periods within sequences which are realizations of independent random variables for different sequences. Therefore, assuming normal distributed sample means, null-hypotheses (that the effects are 0) can be tested with the usual t-test for differences in expected values (between sequences) of the independent samples of sums or differences between periods of the individual data. To test the treatment effect (α=0), the differences between period 1 and 2 in the sample with sequence tiopronin-placebo is compared with those in the sample with sequence placebo-tiopronin with the t-test. To test the period effect (β=0), the differences between period 2 and 1 are compared with the t-test between sequences, and to test the interaction (γ=0), the sums of the periods are compared between the sequences [1].

4 RESULTS

Main parts of the results have been analysed together with Prof. em. Berthold Schneider. (see 3.5).

4.1 DATA SETS ANALYSED

The study was performed with 13 volunteers, 6 females and 7 males. All fulfilled the inclusion criteria, had no exclusion criteria and finished both periods. The statistics include data sets of all 13 subjects for all efficacies and all safety analysis from both periods.

One missing blood alcohol data of subject no. 14 in period 1 at 4 p.m. is related to an adverse event (event 3 in table 17).

4.2 DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

The statistics of the body data (age, body height and weight, blood pressure, pulse rate, temperature, BMI – see 3.4.1.1 and 3.4.1.2) are shown in table 8. The average age was 23, with a minimum of 18 and a maximum of 30 years. Vital parameters and temperature were physiological. The BMI varied widely between 19.78 and 28.38 with an average value at 23.84.

Sequ.	Subj. No.	Init.	Sex	Age	Systolic blood pressure	Diastolic blood pressure	Pulse	Temp.	BMI
A-B	1	ME	male	22	110	65	71	36.5	28.41
	2	CP	female	21	125	70	72	37.2	20.82
	5	LB	female	23	110	65	60	36.8	22.83
	8	MN	male	22	125	80	68	36.8	20.59
	12	JS	female	22	120	75	64	36.4	19.78
	14	JV	female	18	130	80	68	37.2	28.38
	Mean			21.33	120	72.5	67.17	36.82	23.47
B-A	3	PC	male	26	130	70	69	36.9	22.69
	4	CB	male	28	115	55	68	36.6	28.08
	6	PM	female	23	105	65	64	36.3	21.63
	7	FS	male	26	125	85	58	37	26.67
	10	AN	female	23	115	70	56	36.7	27.39
	11	JW	male	18	115	75	68	37.2	20.43
	13	GS	male	30	110	75	56	36.3	22.19
	Mean			24.86	116.43	70.71	62.71	36.71	24.15
	Total mean			23.23	118.08	71.54	64.77	36.76	23.84
	Min.			18	105	55	56	36.3	19.78
	Max			30	130	85	72	37.2	28.38

Table 8: Statistics of age, body measures, blood pressure and temperature

6 of the volunteers were treated in the first period with tiopronin and in the second with placebo (sequence A-B), 7 in the first period with placebo and in the second with tiopronin (sequence B-A).

None of the female subjects was pregnant; 4 used oral contraception and 2 other sufficient contraception. All volunteers were Caucasians, 12 non-smokers and 1 sometimes smoker, who did not smoke during the study period.

Medical history showed for 9 volunteers no diagnosis, 2 migraine, 1 cruciate ligament surgery both sides 2013 and 1 pneumonia in childhood; 10 had no surgeries, 1 left wrist

(healed), 1 contusio cerebri and 1 spontaneous pneumothorax 2011. No regular medication was used by 9 volunteers. In the medication history of the last year 1 subject used metoprolol 50 mg/day, 1 subject amoxicillin 750 mg/day 10/09-12/09, 1 subject sumatriptan 50mg/day and 1 subject budenosid 1-0-1/day 2012- 08/2015. All subjects took the last concomitant medication at least 4 weeks before the study started.

The physical examination (see 3.4.1.2) showed no abnormal findings in 9 volunteers, acne in 2, tattoos in 1 and an old scar on the right thorax in 1 volunteer. There were no abnormal ECG findings. 7 volunteers had no abnormal laboratory findings. The principle investigator decided on a case by case basis which slight abnormal laboratory findings were acceptable. Clinical acceptable findings were: slightly increased uric acid, increased thrombocytes and CK (related to sport injury), slightly increased blood sedimentation rate, slightly increased hemoglobin, slightly increased erythrocytes in one and slightly increased leucocytes in two volunteers. All data is available in the TMF of the principal investigator Dr. Dr. med. Michael Kroll.

4.3 PRIMARY OBJECTIVE

4.3.1 Alcohol

In each period, all volunteers received one hour after application of the test drug 0.8 g/kg body weight pure alcohol in form of 40 % Vodka. Blood was drawn before (but after application of the drugs) alcohol consumption (time 0) and 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h after alcohol consumption and the alcohol concentration was measured (see 3.4.2.1).

Subj. No	Seq	Alcohol concentration (‰) in period 1										
		0 h	0.25 h	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6h	8 h	12 h
1	A-B	0.0	0.4	1.3	1.2	1.1	1.0	0.8	0.6	0.2	0.0	0.0
2	A-B	0.0	0.2	0.6	0.9	0.9	0.8	0.7	0.6	0.3	0.0	0.0
3	B-A	0.1	0.8	0.9	0.9	0.8	0.8	0.6	0.5	0.2	0.0	0.0
4	B-A	0.0	0.3	0.6	1.0	1.1	1.0	0.8	0.6	0.2	0.0	0.0
5	A-B	0.0	0.2	0.9	1.1	1.0	0.9	0.7	0.5	0.0	0.0	0.0
6	B-A	0.0	0.2	0.6	1.2	1.0	1.0	0.8	0.6	0.1	0.0	0.0
7	B-A	0.0	0.2	0.7	1.1	0.9	0.8	0.6	0.5	0.1	0.0	0.0
8	A-B	0.0	0.3	1.1	1.0	0.9	0.8	0.7	0.5	0.2	0.0	0.0
10	B-A	0.0	0.4	0.9	1.2	1.1	1.0	0.8	0.5	0.0	0.0	0.0
11	B-A	0.0	0.1	0.2	0.5	0.8	0.8	0.6	0.5	0.2	0.0	0.0
12	A-B	0.0	0.2	0.5	0.8	0.9	0.8	0.6	0.3	0.0	0.0	0.0
13	B-A	0.1	0.3	0.5	0.8	0.9	0.8	0.6	0.4	0.0	0.0	0.0
14	A-B	0.0	0.6	1.2	1.2	1.1	1.0	0.8	0.6	0.2	0.0*	0.0
Mean		0.0	0.3	0.8	1.0	1.0	0.9	0.7	0.5	0.1	0.0	0.0

Table 9: Individual alcohol concentration period 1
*=missing data (see 4.1), replaced by the mean of the non-missing data

Subj. No	Seq	Alcohol concentration (‰) in period 2										
		0 h	0.25 h	0.5 h	1 h	1.5 h	2 h	3 h	4 h	6h	8 h	12 h
1	A-B	0.0	0.4	0.8	1.2	1.1	1.0	0.8	0.6	0.3	0.0	0.0
2	A-B	0.0	0.8	0.7	1.2	1.2	1.1	1.0	0.9	0.5	0.0	0.0
3	B-A	0.0	0.4	0.8	1.0	0.9	0.8	0.7	0.5	0.2	0.0	0.0
4	B-A	0.0	0.8	1.2	1.3	1.1	1.0	0.8	0.6	0.3	0.0	0.0
5	A-B	0.0	0.6	0.8	1.2	1.1	1.0	0.8	0.7	0.3	0.0	0.0
6	B-A	0.0	0.2	0.6	1.3	1.2	1.0	0.9	0.6	0.1	0.0	0.0
7	B-A	0.0	0.2	0.7	1.0	1.0	0.8	0.7	0.5	0.1	0.0	0.0
8	A-B	0.0	0.6	1.2	1.1	1.0	0.9	0.7	0.5	0.2	0.0	0.0
10	B-A	0.0	0.5	0.9	1.4	1.0	1.0	0.8	0.6	0.1	0.0	0.0
11	B-A	0.0	0.3	0.5	0.5	0.8	0.8	0.7	0.5	0.2	0.0	0.0
12	A-B	0.0	0.4	0.9	1.1	1.0	0.9	0.6	0.4	0.0	0.0	0.0
13	B-A	0.0	0.2	0.4	0.8	0.9	0.8	0.7	0.5	0.1	0.0	0.0
14	A-B	0.0	0.3	0.6	1.0	1.1	1.0	0.8	0.6	0.1	0.0	0.0
Mean		0.0	0.4	0.8	1.1	1.0	0.9	0.8	0.6	0.2	0.0	0.0

Table 10: Individual alcohol concentrations period 2

All 13 subjects reached the primary endpoint and provided pharmacokinetic profiles in both periods. Values of individual alcohol-time concentrations are shown in table 9 and 10.

According to the Widmark formula males should have an estimated peak blood alcohol concentration of 1.14 ‰ and females of 1.33 ‰. Nearly all subjects reached the calculated blood alcohol concentration apart from subject 11 (male) and subject 13 (male), who's highest values were 0.8 ‰ resp. 0.9 ‰.

Figures 7 and 8 present the blood alcohol concentrations over the time for all subjects after taking tiopronin and after taking placebo. The red line shows the average course with a quite similar course in both groups.

The pharmacokinetic profiles of ethanol show a rapid increase in concentration, reaching the maximum (C_{max}) at about 1 hour (t_{max}) after alcohol administration. These values are mainly determined by the absorption of ethanol. The second part of the curves shows a slow, almost linear decrease. Zero values are measured at least 8 hours after alcohol intake in every subject. This part of the graph reflects mainly the elimination of ethanol.

Pharmacokinetic profiles of every participant are graphically represented in appendix 10.6.1.

On the significance level 0.05, the elimination rate k, t_{max} and C_{max} was not affected by the treatment, the period or interaction effects. The AUC was not influenced by treatment or interaction, but was slightly significant (p=0.026) in the second period (see red mark in table 11). This effect may be explained by random fluctuations [1].

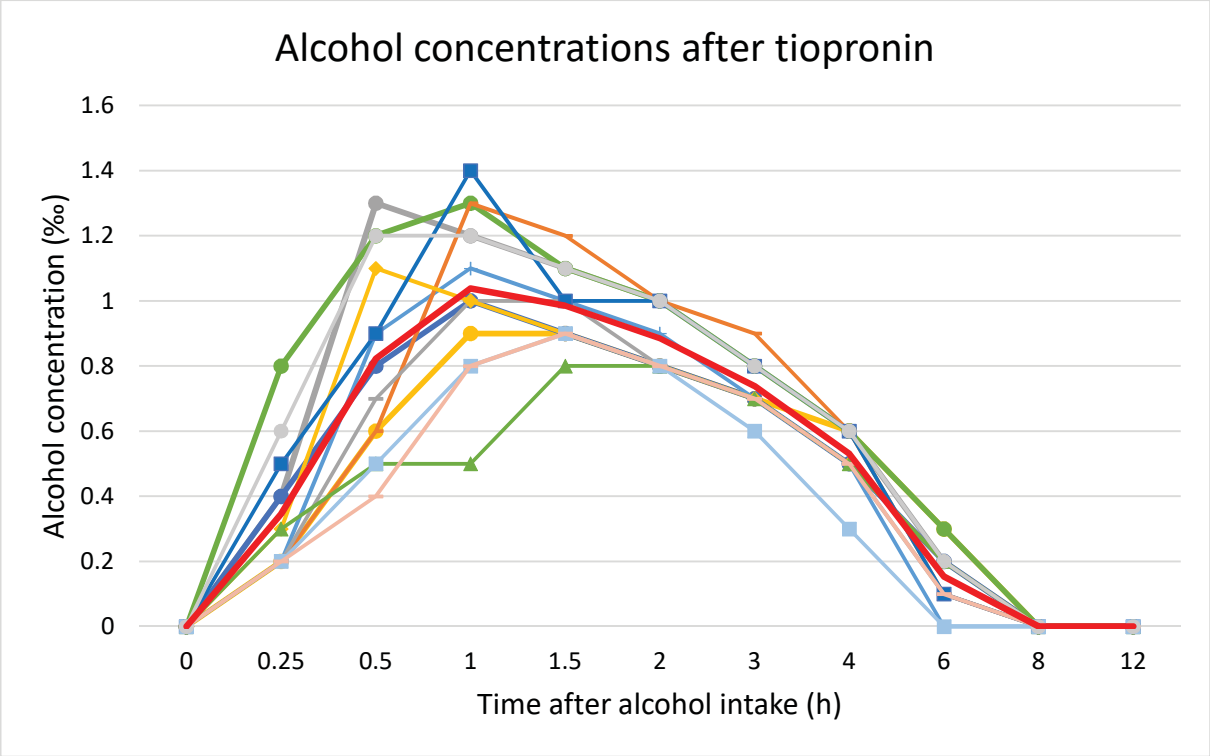

Fig. 7: Time course of alcohol concentration after tiopronin intake [1]
The red line shows the grand mean

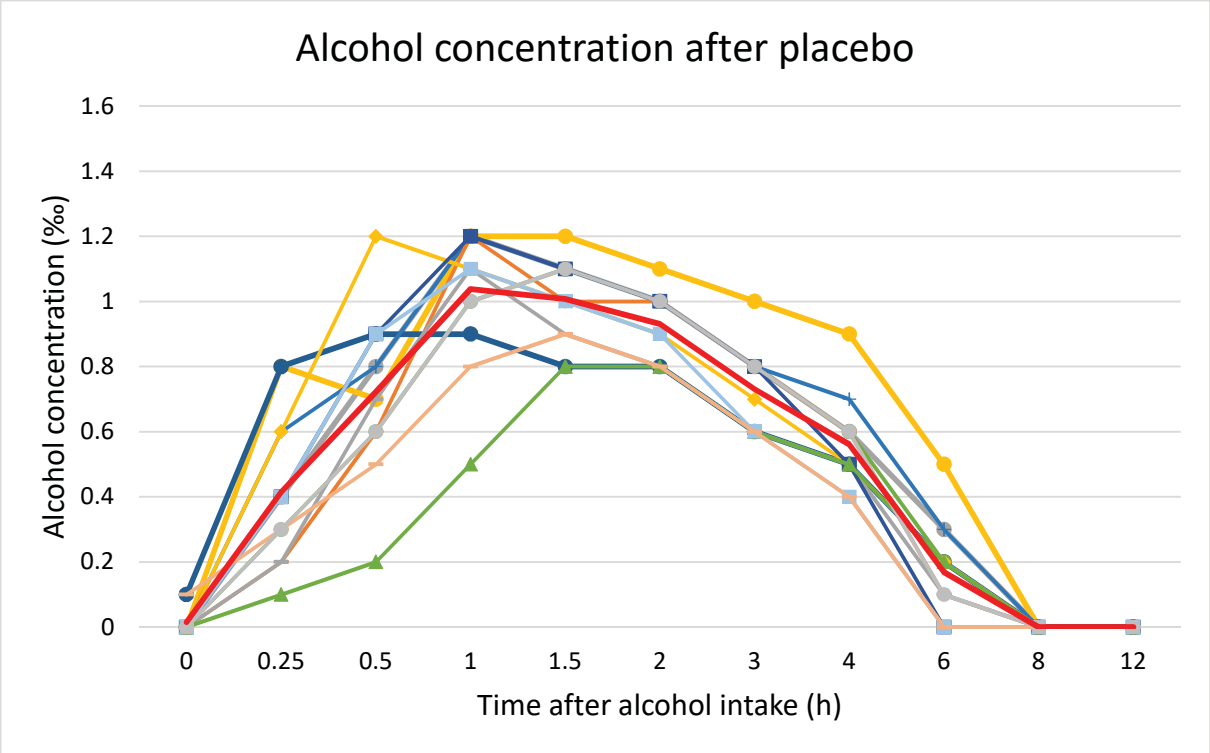

Fig. 8: Time course of alcohol concentration after placebo intake [1]
The red line shows the grand mean

Table 11 shows grand mean, treatment, period and interaction effects for all estimated kinetic parameters.

Kinetic Parameter	Effect name	Effect size	Standard error	95% Confidence Interval		Sign. p
				lower	upper	
t_{max} [h]	grand mean	1.02976	0.07574	0.86306	1.19646	<0.001
	treatment	-0.04167	0.05678	-0.16664	0.08331	0.478
	period	-0.04167	0.05678	-0.16664	0.08331	0.478
	interaction	-0.1131	0.07574	-0.02798	0.05361	0.164
C_{max} [‰]	grand mean	1.09464	0.04255	1.001	1.18829	<0.001
	treatment	-0.00298	0.01888	-0.04452	0.03857	0.878
	period	-0.03869	0.01888	-0.008024	0.00286	0.065
	interaction	0.03036	0.04255	-0.06329	0.124	0.49
AUC [‰*h]	grand mean	3.97649	0.16051	3.62321	4.32977	<0.001
	treatment	-0.06548	0.08559	-0.25386	0.12291	0.46
	period	-0.21994	0.08559	-0.40833	-0.03155	0.026
	interaction	0.206	0.16051	-0.15268	0.55387	0.237
Linear Elimination k [‰/h]	grand mean	0.19295	0.00957	0.17188	0.21402	<0.001
	treatment	0.00092	0.0026	-0.0048	0.00664	0.729
	period	-0.00225	0.0026	-0.00797	0.00347	0.405
	interaction	-0.00213	0.00957	-0.0232	0.01894	0.828

Table 11: Estimated effects for kinetic parameters [1]
The red marked area shows the slight significant period effect in AUC values.

Table 12 shows grand mean, treatment, period and interaction effects for all estimated kinetic parameters for individuals and sequences. Based on table 12 AUC, elimination rate k, t_{max} and C_{max} are graphically presented in figure 9 to 12.

Subject number	Period 1				Period 2			
	t_{max}	C_{max}	AUC	k	t_{max}	C_{max}	AUC	k
1	0.5	1.3	4.59	0.2	1	1.2	4.6	0.18
2	1	0.9	3.97	0.13	1	1.2	5.84	0.16
5	1	1.1	3.56	0.2	1	1.2	4.81	0.18
8	0.5	1.1	3.89	0.16	0.5	1.2	4.18	0.18
12	1.5	0.9	2.74	0.24	1	1.1	3.36	0.23
14	0.5	1.2	4.6	0.2	1.5	1.1	4	0.22
Mean A−B	0.8333	1.0833	3.8917	0.1895	1	1.1667	4.4625	0.1922
3	0.5	0.9	3.75	0.14	1	1	3.8	0.16
4	1.5	1.1	4.2	0.2	1	1.3	4.9	0.2
6	1	1.2	4.02	0.22	1	1.3	4.27	0.24
7	1	1.1	3.46	0.2	1	1	3.56	0.2
10	1	1.2	3.89	0.23	1	1.4	4.31	0.26
11	1.5	0.8	3.1	0.15	1.5	0.8	3.36	0.15
13	1.5	0.9	2.93	0.2	1.5	0.9	3.3	0.18
Mean B−A	1.1429	1.0286	3.6214	0.1919	1.1429	1.1	3.9304	0.1983
Total mean	1	1.0539	3.7462	0.1908	1.0769	1.1308	4.176	0.1954

Table 12: Estimated effects of kinetic parameters for individuals and sequences

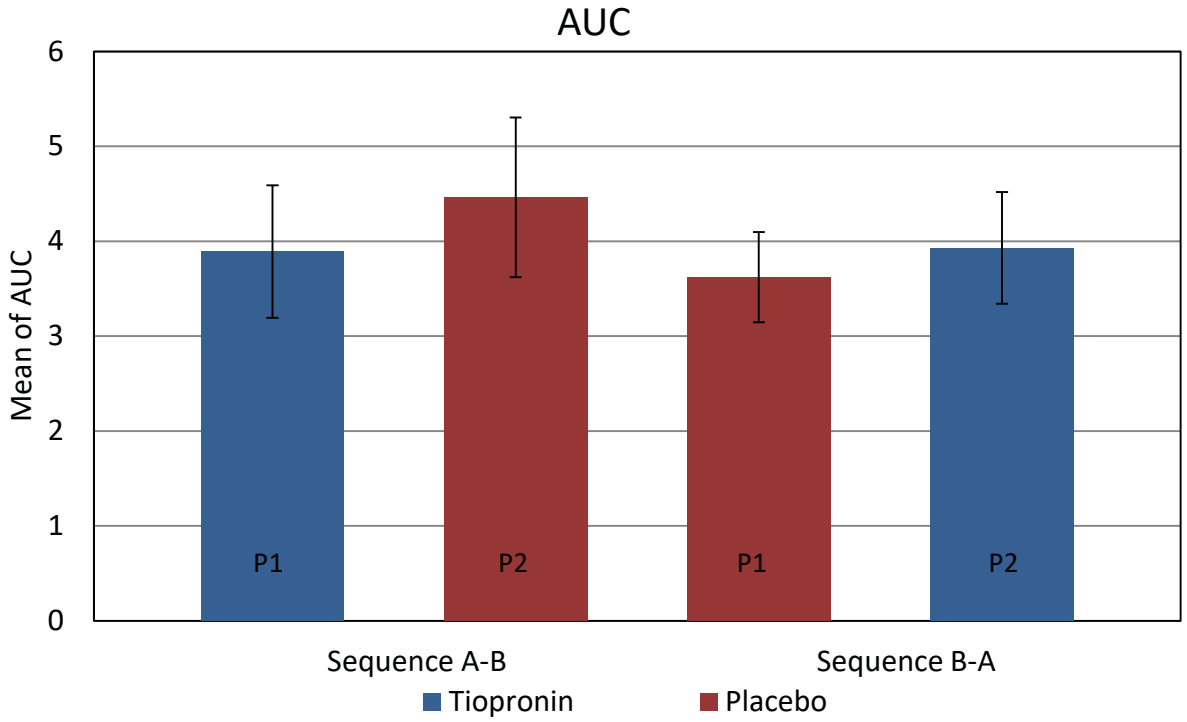

Fig. 1: Mean of AUC [1]
P1=period 1, P2=period 2

The bars in fig. 9 show mean of AUC in different periods and after different treatments. The AUC values do not differ significantly between different treatments. Highest and lowest mean refer to the placebo treatment, while means after tiopronin treatment lie midway between the two values.

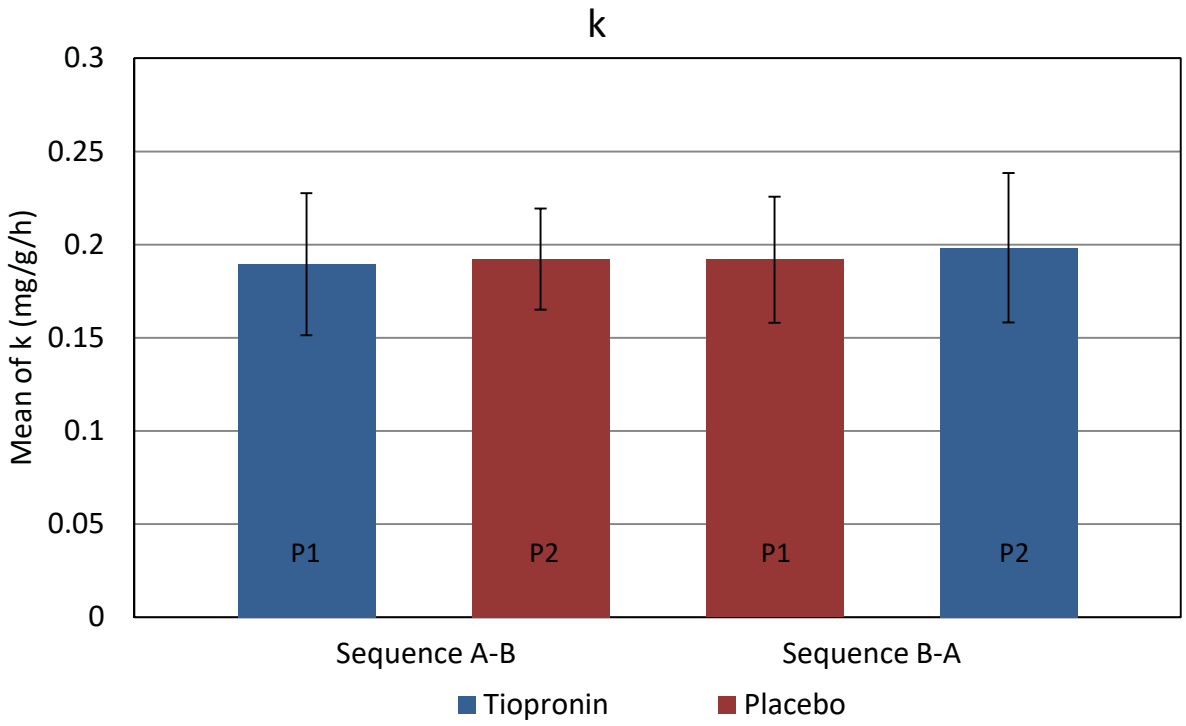

Fig. 2: Mean of elimination rate k [1]
P1=period 1, P2=period 2

The bars in fig. 10 represent the mean of k in different periods and after different treatments. Elimination rate k differs between 0.189 and 0.198 mg/g/h. All bars are equal in height. There was no difference in treatment or periods.

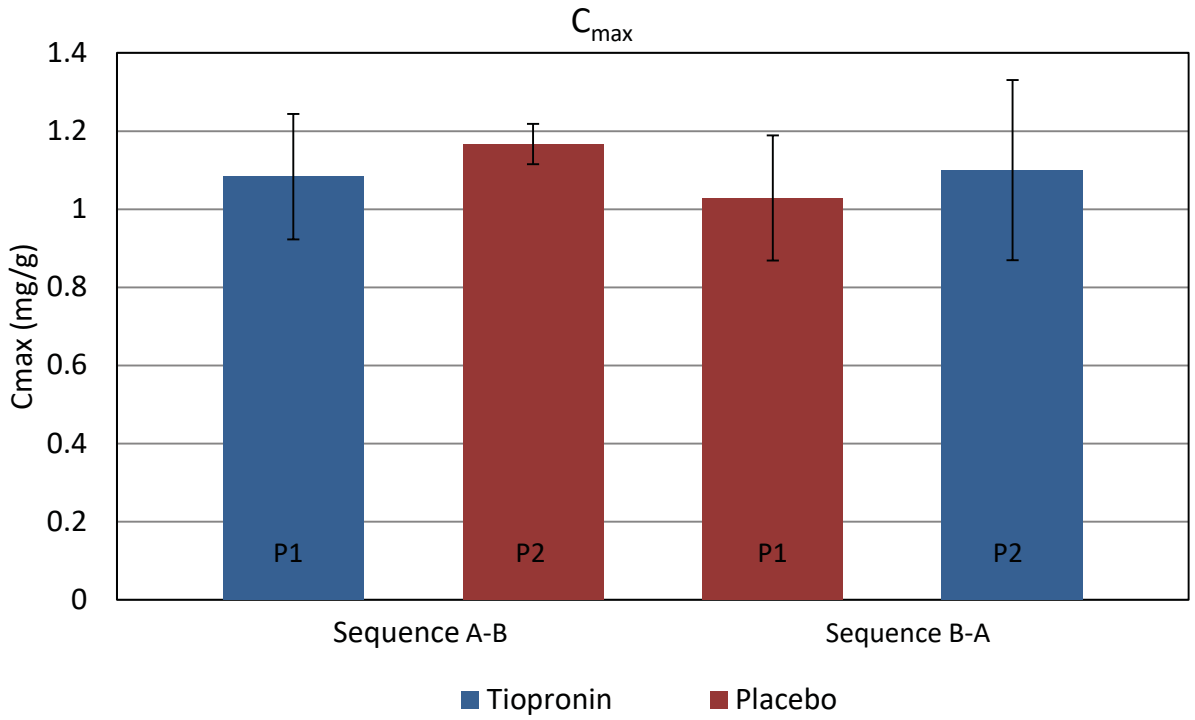

Fig. 3: Mean of estimated C_{max}
P1=period 1, P2=period 2

The bars in fig. 11 show means of the maximal alcohol concentration in different periods and after different treatments. All means lay in between 1 mg/g and 1.2 mg/g. The highest and lowest values occur after the treatment with placebo. We did not see a treatment or a period effect.

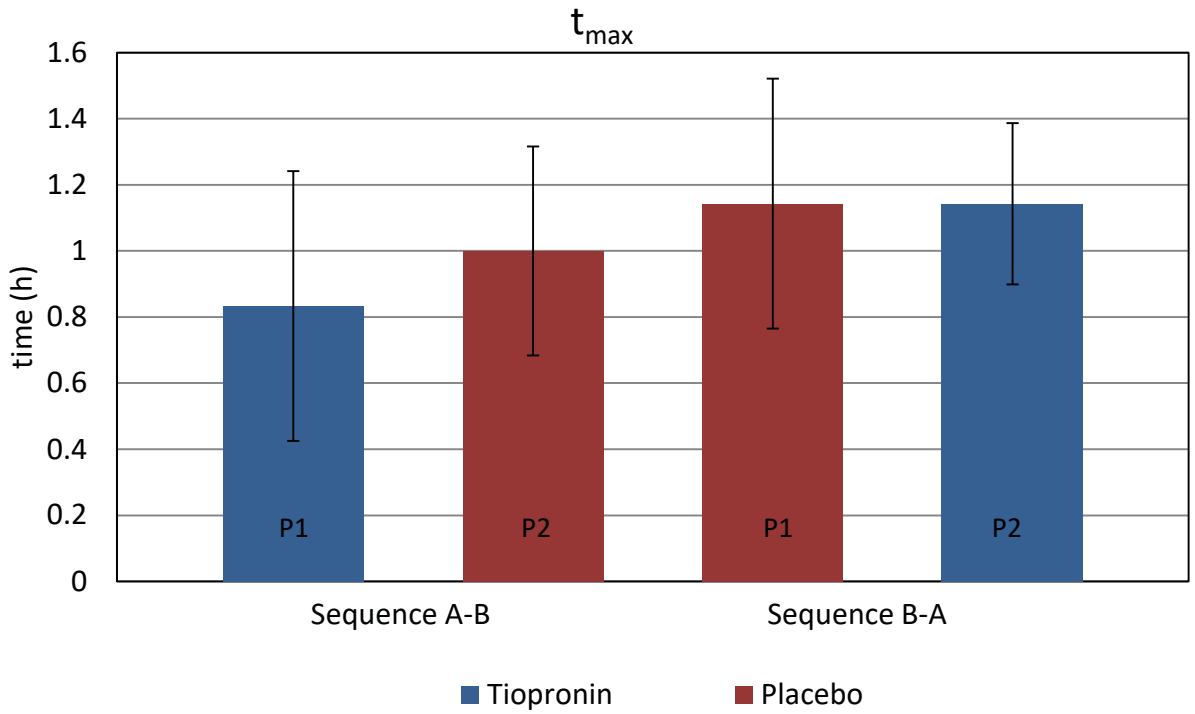

Fig. 4: Mean of estimated t_{max}
P1=period 1, P2=period 2, t_{max} = ıime when estimated maximum concentration is achieved.

The bars in fig. 12 show the mean of the time when the estimated maximum concentration was achieved. Sequence A-B shows in the first period after tiopronin intake the lowest mean of t_{max}. That means that the average of subjects in sequence A-B reached their maximum concentration in period 1 already rounded 50 min after alcohol intake and in period 2, 1 h after alcohol intake. sequence B-A has generally a longer absorption phase and reaches maximum concentrations 1.14 h or 68 min after alcohol intake in both periods. Standard deviations range widely in between different subjects of one sequence. Means of placebo and tiopronin do not differ significantly. Means of different periods do not differ significantly. As conclusion, the maximum concentration is achieved differently in between sequences, but there was no treatment or period effect found.

Knowing that the distribution and elimination of alcohol depends on age, sex and BMI [26], the hypothesis, whether the expected values (means) of the estimated kinetic parameters do not differ between bivariate categories of the covariates gender (female, male), age (< 25 years, ≥ 25 years) and BMI (< 25 kg/m2, ≥ 25 kg/m2) was additionally tested. Using a t-test for independent samples no differences in the covariates were found. The results are shown in table 32-34 of appendix 10.6.2.

4.4 SECONDARY OBJECTIVES

4.4.1 Acetaldehyde

The acetaldehyde measurements (in µ g/ml serum) were performed before (time 0 h), 2 h and 4 h after alcohol consumption in both periods (see 3.4.2.2). The results are given (together with the means within sequence and period) in table 13.

Sequ.	Subject number	Period 1			Period 2		
		Acetaldehyde	Acetaldehyde	Acetaldehyde	Acetaldehyde	Acetaldehyde	Acetaldehyde
		0h	2h	4h	0h	2h	4h
A-B	1	0.00	0.08	0.15	0.02	0.04	0.09
	2	0.02	0.58	0.84	0.00	0.24	0.25
	5	0.05	2.25	1.01	0.00	1.25	1.17
	8	0.01	0.47	0.57	0.03	0.59	0.81
	12	0.00	1.07	1.35	0.04	1.10	1.52
	14	0.00	1.08	0.93	0.00	0.59	0.41
	Mean	0.01	0.92	0.81	0.02	0.63	0.71
	Standard deviation	0.02	0.75	0.41	0.02	0.47	0.56
B-A	3	0.02	0.53	0.41	0.00	0.72	0.76
	4	0.00	0.45	0.32	0.00	0.41	0.31
	6	0.06	1.14	1.22	0.01	0.77	1.09
	7	0.00	0.55	0.46	0.10	0.67	1.55
	10	0.00	0.52	0.57	0.03	1.11	0.93
	11	0.00	0.96	0.83	0.08	0.55	0.63
	13	0.00	0.26	0.26	0.00	0.44	0.40
	Mean	0.01	0.63	0.58	0.03	0.67	0.81
	Standard deviation	0.02	0.31	0.34	0.04	0.24	0.43

Table 13: Mean and individual acetaldehyde concentrations in µg/ml serum
Including standard deviation (SD)

Table 13 shows the estimated effects of tiopronin on the concentration of blood acetaldehyde with standard error, 95% confidence interval and p value of the t-test. Treatment effect, period effect and interaction do not differ significantly from 0. Acetaldehyde is not influenced by tiopronin, and it is similar in both periods. Furthermore, the intra-individual standard deviation for acetaldehyde varies from nearly 0 µg/ml serum to 0.76 µg/ml serum (table 13, subject 7). Inter-individual standard deviations are particularly high with 0.75 in sequence A-B, period 1 at time-point 2 h.

The means of acetaldehyde concentrations are graphically represented in figure 13 and 14.

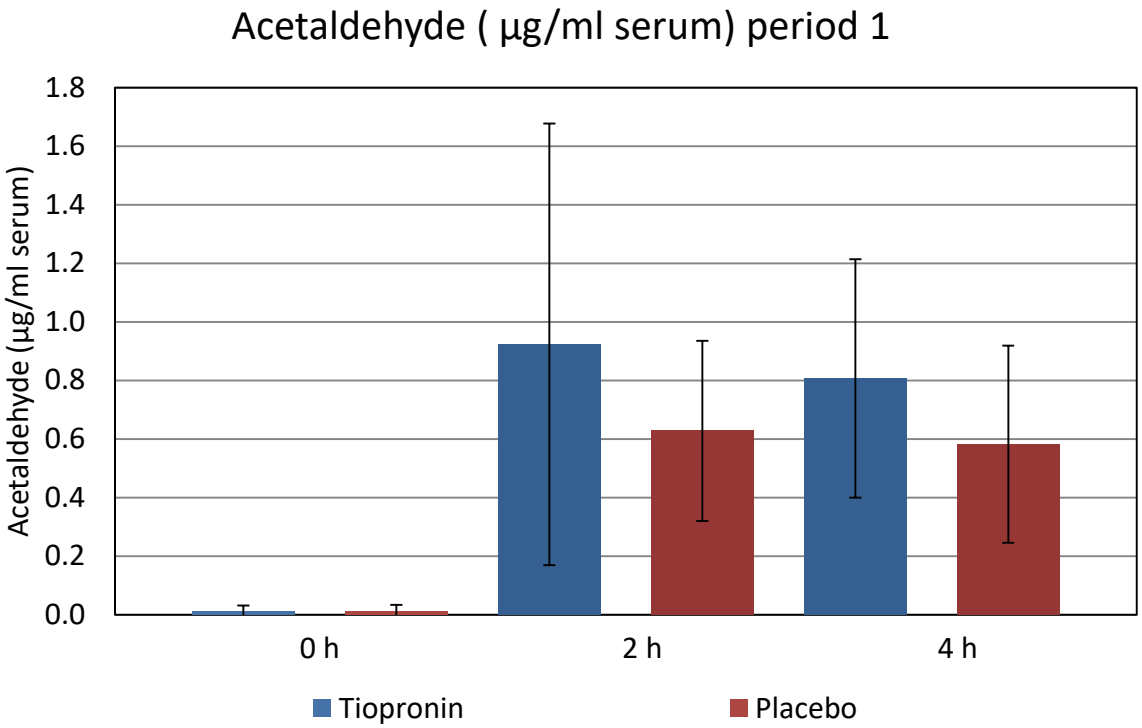

Fig. 5: Mean of acetaldehyde concentrations in period 1

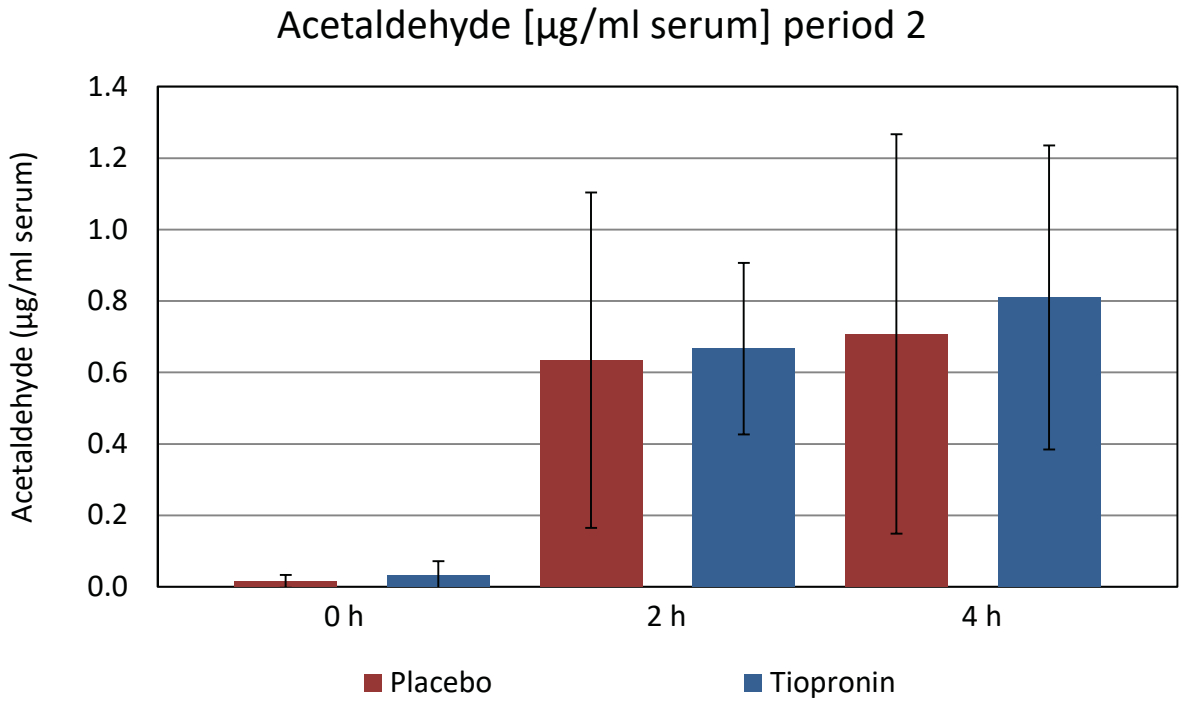

Fig. 6: Mean of acetaldehyde concentrations in period 2
Standard deviation differs widely in between subjects

The graphs show the mean concentration of acetaldehyde values in the first and second period, after different treatments. At time-point 0 all values are close to 0 µ g/ml serum. Mean maximum concentrations were found between 0.8 and 1 µg/ml serum, but differ widely between subjects (figure 13, 14 and table 13). Concentrations of acetaldehyde are still increasing 4 h after alcohol consumption in the second, but not in the first period. No statistical significant differences in treatment or period could be found [1].

4.4.2 Number combining test (ZVT)

In each period a number combining test was performed by each volunteer before (time 0 h) and 2 h after alcohol consumptions (see 3.4.2.3). The results are given (together with the means within sequence and period) in table 14.

ZVT {bits/sec]	Effect name	Effect size	Standard error	95% Confidence Interval		Sign. p
				lower	upper	
0 h	grand mean	2.8674	0.0886	2.6726	3.0623	<0.001
	treatment	0.0222	0.0244	-0.0315	0.0759	0.382
	period	-0.2864	0.0244	-0.34	-0.2327	<0.001
	interaction	0.0317	0.0886	-0.1632	0.2266	0.727
2 h	grand mean	2.9527	0.0714	2.7955	3.1098	<0.001
	treatment	0.0047	0.026	-0.0526	0.062	0.86
	period	-0.1664	0.026	-0.2236	-0.1091	<0.001
	interaction	-0.0077	0.0714	-0.1648	0.1495	0.916
Diff. 2-0 h	grand mean	0.0852	0.0345	0.0094	0.1611	0.031
	treatment	-0.0175	0.0274	-0.0778	0.0428	0.536
	period	0.12	0.0274	0.0597	0.1803	0.001
	interaction	-0.0394	0.0345	-0.1153	0.0365	0.277

Table 14: Estimated effects for ZVT

The mean ZVT increases significantly in the first period between 0 h and 2 h from 2.64 to 2.78 bits/sec after tiopronin treatment and from 2.53 to 2.79 bits/sec after placebo treatment. In the second period the mean ZVT is significantly higher than in the first and decreases only slightly but not significantly between 0 and 2 h. There were no significant differences between both treatments. The means are graphically represented in figure 15.

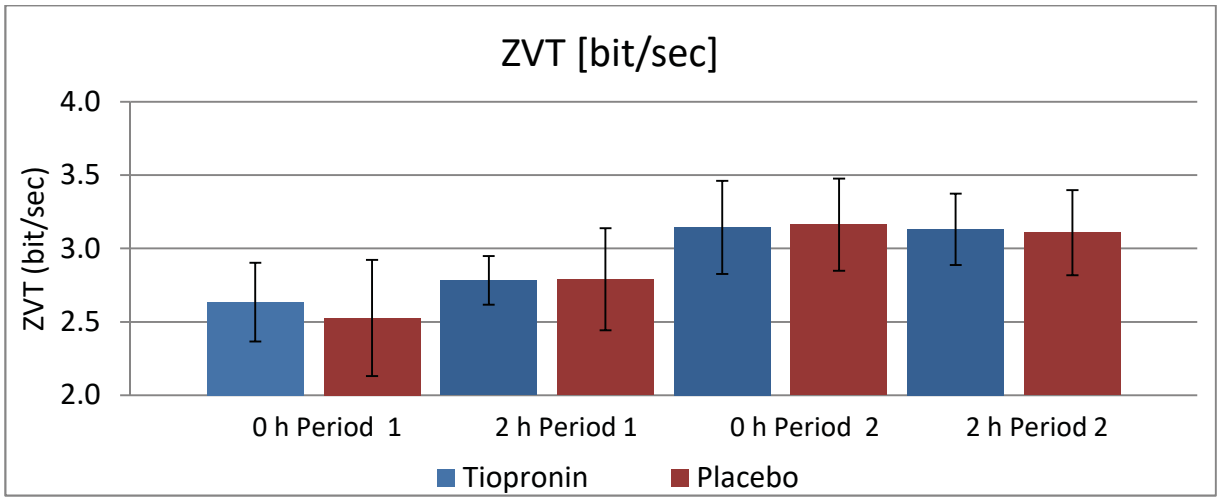

Fig. 7: Means of results in ZVT in both treatments and periods [1]

Fig. 15 shows the means of perceptual speed measured in bit/sec for each treatment in each period. The average performance increased in the first period and in between the first and the second period. Values over 3 bit/sec were only achieved in the second period, but did not further improve during this period.

A table with individual results is available in appendix 10.6.3.

Summarising all results from the ZVT there was a period, but not a treatment or interaction effect [1].

4.4.3 Breath alcohol concentration (Dräger Alcotest 6810)

In each period the alcohol concentration in breath was measured with Dräger Alcotest 6810 before alcohol intake (time 0 h) and 1 h after alcohol intake (see 3.4.2.4). The individual data and means within sequence and period are shown in appendix 10.6.4. All measurements before alcohol intake were 0.

The difference in the means of the measurements 1 h after alcohol intake between sequences within periods were tested with t-test for independent data, the difference in the means between periods within volunteers with the paired t-test. The results are shown in table 15.

Dräger Alcotest 6810 1h mg/l	Sequence	Mean	Standard deviation	Mean Difference	Standard error	95% Confidence Interval		Sign. p 2-sided
						lower	upper	
Period 1	A-B	0.77	0.0827	0.0386	0.0645	-0.1034	0.1806	0.562
	B-A	0.7314	0.1377					
Period 2	A-B	0.8033	0.0864	0.0105	0.0675	-0.1382	0.1591	0.88
	B-A	0.7929	0.1442					

Dräger Alcotest 6810 1h mg/l	N	Mean	Standard deviation	Mean Difference	Standard error	95% Confidence Interval		Sign. p 2-sided
						lower	upper	
Period 1	13	0.7492	0.1128	-0.0485	0.0316	-1174	0.0204	0.151
Period 2	13	0.7977	0.1163					

Table 15: Results of t-test for differences in breath alcohol concentrations [1]

P-values of the treatment effect (p=0.562 in period 1, p=0.88 in period 2) and of the period effect (p= 0.151) are always greater than 0.05, therefore measured values of breath alcohol concentration are not significantly greater or smaller from the estimated distribution. There were no significant differences in breath alcohol concentration for treatment or periods.

To investigate the relation between the results of Dräger Alcotest 6810 and serum blood alcohol concentration 1 h after intake, a linear regression line was fitted to both data in period 1 und 2 by least square method. The connection between both measurements is in the first period (correlation r=0.665, regression coefficient b=0.357, p=0.013) weaker and the regression more flat than in the second one (correlation r=0.843, regression coefficient b=0.413, p<0.001). There is no consistent linear regression found in-between breath and blood alcohol concentration [1].

4.4.4 Safety evaluation

4.4.4.1 General safety evaluation

At the beginning of each period the accordance of the study performance with the protocol was checked. All patients were fastening and had had no alcohol consumption. During the study periods the investigational product (tiopronin or placebo) and alcohol (0.8 g/kg body weight) were applied according to the protocol.

For safety control, vital parameters (blood pressure, pulse rate, oxygen saturation) were monitored during the study periods every 4 hours (see 3.4.1.1). The statistics of these measurements are shown in table 16.

Period 1	Mean	Std. Dev.	Minimum	Maximum	Valid N
Syst. blood pressure 6:15 am	122.31	9.49	105	140	13
Diast. blood pressure 6:15 am	75.00	6.12	65	85	13
Pulse rate 6:15 am	68.31	5.99	56	76	13
Syst. blood pressure 10:15 am	113.08	13.16	100	140	13
Diast. blood pressure 10:15 am	75.38	7.76	60	90	13
Pulse rate 10:15 am	70.85	10.22	61	98	13
Oxygen saturation 10:15 am	97.77	1.01	96	99	13
Syst. blood pressure 2:15 pm	117.31	13.48	95	140	13
Diast. Blood pressure 2:15 pm	68.08	9.02	50	80	13
Pulse rate 2:15 pm	86.85	14.72	62	103	13
Oxygen saturation 2:15 pm	97.92	.64	97	99	13

Period 2	Mean	Std. Dev.	Minimum	Maximum	Valid N
Syst. blood pressure 6:15 am	116.15	8.20	100	130	13
Diast. blood pressure 6:15 am	70.77	8.38	55	80	13
Pulse rate 6:15 am	64.92	5.69	56	80	13
Syst. blood pressure 10:15 am	106.54	7.47	95	120	13
Diast. blood pressure 10:15 am	66.15	8.45	55	80	13
Pulse rate 10:15 am	77.46	12.39	57	97	13
Oxygen saturation 10:15 am	97.54	1.13	95	99	13
Syst. blood pressure 2:15 pm	112.31	9.04	100	130	13
Diast. Blood pressure 2:15 pm	67.31	6.96	60	80	13
Pulse rate 2:15 pm	92.38	15.45	73	118	13
Oxygen saturation 2:15 pm	97.69	1.03	96	99	13

Table 16: Vital parameters of the volunteers

All values of previous planned measured vital parameter were physiological. One female subject had a systolic blood pressure under 90, which was measured in regards to adverse event 2 (see table 17).

4.4.4.2 Adverse events

All in all there were seven adverse events (for definition and documentation see 3.4.1.4). All events were reversible. No event was serious.

a) AEs during study days

Three adverse events took place on the 14.11.15.

- unspecific pinch in the kidney regions at 08:40 am (male subject)

A young man expressed a slight pinch in both kidney regions. He had no pain and no other concomitant symptoms, so no action was taken. He kept in closer observation until the feeling disappeared after 80 minutes.

- syncope 11:00 am (female subject)

A young woman fainted after a blood draw. The permanent venous access was closed so that a separate puncture was obtained. The subject fainted when she straightened up, and she was carried safely on a bench, where she rested until she was able to walk (30 min). Her systolic blood pressure dropped shortly under 90, but recovered after a view minutes. The subject had no further complaints for the rest of this and the next period.

- headache, nausea and vomiting 04:00 pm (female subject)

A young woman suffered from headache with concomitant nausea and vomiting. She received 30 drops of metamizole (= 750 mg metamizole) one tablet of paracetamol (500 mg), slept and felt better 1 hour later. The blood draw at 04:00 pm was skipped. The next blood planned draw at 08:00 pm took place on request of the subject. The subject felt healthy at the end of the study day and during the next week, so that she was able to participate in the second period. Blood and urine levels from the post-study visit two weeks later were normal.

A fourth AE occurred on the second study day (21.11.15).

- vertigo at 4:00 pm (female subject)

The vertigo ended after 5 min in lying position. No further actions were taken. Blood-test, urine-tests and results from clinical examination in the post-study visit were unsuspicious.

b) AEs post study visit

Three AEs were registered during the post-study visit.

- 25.11.2015: One male subject had atypical lymphocytes. The laboratory suggested an asymptomatic mononucleosis. A follow up test showed normal blood cells (21.01.2016).

- 03.12.2015: One female subject had increased erythrocyte- and leucocyte- urine levels. Additional medication (antibiotics) was prescribed. A follow up test two days later at her family doctor was normal.

- 10.12.2015: One female subject had increased granulocytes and ESR in the post-study evaluation. The values remained increased in the second blood test. The principal investigator documented nasalization but no other clinical symptoms. He prescribed penicilline 1 Mio. units three times daily for 6 days. A third test was taken on 03.02.2016. The amount of granulocytes was normal, but the amount of eosinophil cells was slightly increased. The subject was required to undergo a fourth blood test if any clinical symptom would appear. The subject stated towards the monitor to feel fine and free of symptoms on 04.04.2016.

An overview of all occurred AEs is given in table 17.

Event	Subject	Mild		Moderate		Tiopronin	Placebo
		NR	U	NR	U		
Event 1	1		x			x	
Event 2	6			x			x
Event 3	14				x	x	
Event 4	2		x				x
Event 5	4	x					
Event 6	10	x					
Event 7	5	x					
NR= not related, U= unknown							
Event 1 = unspecific pinch in the kidney regions, Event 2 = fainting during blood drawn, Event 3 = headache, vomiting, Event 4 = vertigo, Event 5 = atypical lymphocytes (mononucleosis), Event 6 = cystitis, Event 7 = increased leucocytes and ESR							

Table 17: Adverse events in relation to treatment [1]

Three adverse events are unknown related to the clinical trial, but seem to be rather related to the consumption of alcohol than to the intake of tiopronin, so that no adverse event is probably related to tiopronin intake. No subject had serious adverse events and all adverse events were reversible [1]. The documentation of all AEs is available in appendix 10.7.

These data indicate that the study performance was safe and of high quality in accordance with the study protocol.

5 DISCUSSION

5.1 OBJECTIVES

Many drugs interact with alcohol and alcohol interacts with many drugs for example analgesics, antibiotics, antihistamines, benzodiazepines and many more [45].

This study was the first to analyse the effects of tiopronin in healthy subjects while drinking alcohol. Our results show that tiopronin does not affect the metabolism of alcohol or the safety of healthy subjects. Moreover, taking modern alcohol studies as a role model [26] this trial makes its small contribution to a big historic field of clinical alcohol research starting with Erik M. P. Widmark [27].

5.2 METHODS

5.2.1 Study design

The clinical trial followed a randomised cross-over design, which enables a power of 90% with only 13 subjects to answer the study's objectives. To make more general statements about the metabolism of alcohol in human, which was not the aim of this work, a larger number of participants is required. To give evidence about the influence of Tiopronin on the metabolism of alcohol a randomised, cross-over design with 13 subjects was appropriate.

5.2.2 Alcohol

For the analytics of blood alcohol a clinical enzymatic method was used. Although headspace chromatographic complies the current gold standard, the enzyme analytic is a common reliable method in clinical every day work. In forensic case work headspace chromatographic analysis as well as this enzyme based method are required to give evidence [30]. This means that this evaluation of samples was well suited to constitute the influence of tiopronin on the metabolism of alcohol in healthy volunteers.

Pharmacokinetic profiles of alcohol were obtained for each subject and analysed for treatment, period and interaction effect. All profiles followed the expected 0 order kinetic and were close to the estimated alcohol concentration of 1.1 ‰ or 1.3 ‰. Neither pharmacokinetic parameter such as t_{max}, C_{max}, AUC nor elimination rate was influenced by the administration of tiopronin compared to placebo. That means no treatment effect in any of the obtained data was seen. A period effect was seen isolated and slightly in AUC, which was most likely caused by random fluctuations. As no other clinical study has answered this question before (see 3.1.2), our findings cannot be discussed in comparison to other research. Also, for penicillamine, a medication with partially identical indications and a similar chemical structure, no interaction with alcohol is known [1], [21].

5.2.3 Acetaldehyde

There were high inter-individual standard deviations, which makes it difficult to draw further conclusions. Considering the possibility of increasing values after 4 h, more measurements after this time-point should be performed in future trials. The measurement of acetaldehyde seems to be a challenging, but rewarding task to be further researched in the future.

5.2.4 Number Connection Test (ZVT)

For people over 16 years of age the ZVT is ideally used as individual test with a one-week wash-out period [42]. Values of ZVT were increasing within the first and in between periods. These results refer to a learning effect and demand a longer wash-out period in future studies.

5.2.5 Breath Alcohol

This study utilized Dräger Alcotest 6810, normally established in hospitals as indicator for ethanol intoxications after gynaecological or urological interventions [44]. Therefore the class of accuracy is not comparable with modern breath alcohol intervention studies [46]. Nevertheless Dräger Alcotest 6810 was suitable used to evaluate volunteers' status of drunkenness as safety parameter.

5.2.6 Safety Analysis

Safety analysis gave further evidence, that intake of tiopronin in combination with alcohol has no safety implications on healthy subjects. Between placebo and tiopronin treatment,

there was no differing effect on drunkenness, and there was no differing effect on vital and organ functions, controlled via physical examination, blood- and urine-tests and measurement of blood pressure, pulse rate and oxygen saturation. All seven occurring adverse events were reversible and most probably not related to tiopronin intake.

5.3 Results, Limitations and Literature

Neither pharmacokinetic parameters such as t_{max}, C_{max}, AUC and elimination rate nor acetaldehyde levels nor individual psychometric response were influenced by the administration of tiopronin compared to placebo. That means we did not see a treatment effect in any of the obtained data. Period effects were seen isolated and slightly in AUC, which is probably caused by random fluctuations and in ZVT results. The latter refers to a learning effect and demands a longer wash-out period in future studies. Safety analysis gave further evidence, that tiopronin has no effect in healthy subjects.

In terms of limitations of this study, it has to be considered that results are not representative for long term effects due to regularly alcohol and tiopronin intake. Regarding acetaldehyde measurements, more measuring points should be captured in future studies to give a more precise concentration-time profile.

Results of a similar study of *Hoshino et al.* illustrating that tiopronin may reduce blood alcohol concentration in rabbits [25] are now not only opposed to information about similar drugs as D-penicillamine, they are also contrary to the quintessence of this work. The source of this contradiction can not be answered here. Pharmacokinetic differences between rabbits and humans, differently used methods, unknown ways of metabolic pathways and more reasons are conceivable. A control study in rabbits based on the pattern of *Hoshino et al.* and pre-clinical research could give further information.

However the strength of this research project lies in the value of raised data. In terms of clinical relevance this study gives first evidence that simultaneous administration of alcohol and tiopronin in humans has no influence on the effect of alcohol in the human body. The effect of alcohol towards the level of administrated tiopronin was not measured, but appears to play an ancillary role for cystinuric patients, who are treated with this drug

continuously to prevent stone formation and may consume alcohol only occasionally. Regularly consumption of alcohol is in every case not recommended.

6 CONCLUSION

The topic of this study was the influence of tiopronin on the metabolism of alcohol in healthy subjects.

Summarising all findings, occasional consumption of alcohol during the treatment with tiopronin might be allowed for patients. Nevertheless, it has to be considered that the results are not representative for long term effects due to regular alcohol and tiopronin intake. However, the dangerous nature of alcohol consumption in general should be communicated simultaneously [1].

The first version of the study report was available 9th of April 2016. This report was submitted to the Federal Institute for Drugs and Medical Devices (BfArM). According to law, all collected subject data will be kept for at least 10 years at the study centre. Trial master files from the sponsor and the investigator are available in electronic and printed versions.

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10 APPENDIX

10.1 ETHICS COMMITTEE

004921143022279

ÄRZTEKAMMER NORDRHEIN
Körperschaft des öffentlichen Rechts

ETHIKKOMMISSION

MIT Gesundheit GmbH
Herrn Dr. Josef Karl Merges
Stechbahn 20 – 22
47533 Kleve

20.05.2014 Prof. S.-W./Se

cc: BfArM

Studie nach GCP-V (Monozentrisch)

EudraCT-Nr.: 2014-000059-95
Protokoll-Nr.: MIT-TIO-1312-01
Titel: INFLUENCE OF TIOPRONIN ON THE METABOLISM OF ALCOHOL IN HEALTHY SUBJECTS
Sponsor: MIT Gesundheit GmbH
Antragsteller: MIT Gesundheit GmbH, Dr. Josef Karl Merges, Kleve
LKP: Dr. Dr. med. Michael Kroll, Kleve
Unser Zeichen: 2014060

Sehr geehrter Herr Dr. Merges,

die Ethikkommission hat Ihren oben genannten Antrag in der Sitzung am 11.03.2014 beraten. Die zusätzlich angeforderten Informationen sind am **19.05.2014** eingegangen.

Die Ethikkommission erteilt eine zustimmende Bewertung.

Begründung:
Versagungsgründe gemäß § 42 Abs. 1 AMG liegen nicht vor.

Der Bewertung liegen die in Anhang 1 aufgeführten Unterlagen zugrunde.

Die Bewertung ist für die in Anhang 2 aufgeführten Prüfer, Stellvertreter und Prüfstelle gültig

Ärztekammer Nordrhein
Tersteegenstraße 9 · 40474 Düsseldorf
Telefon 0211/4302-2272 - Telefax 0211/4302-2279
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Düsseldorf (BLZ 300 606 01) 0001 145 290
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Kernarbeitszeit: Montag bis Donnerstag 9 Uhr bis 16 Uhr, Freitag 9 Uhr bis 14 Uhr

004921143022279

ÄRZTEKAMMER NORDRHEIN
Körperschaft des öffentlichen Rechts

ETHIKKOMMISSION

Seite 2 **lfd. Nummer 2014060**

An der Abstimmung haben die in Anhang 3 aufgeführten Kommissionsmitglieder teilgenommen.

Mit freundlichen Grüßen

Prof. Dr. med. H. Schulte-Wissermann
Stellvertr. Vorsitzender
der Ethikkommission

Anlagen:
Anhänge 1 – 3
Berichte zu SUSARs/SAEs

Allgemeine Hinweise:

- Die ethische und rechtliche Verantwortung für die Durchführung dieser klinischen Prüfung verbleibt beim Sponsor, bei der Leiterin/dem Leiter der klinischen Prüfung und bei den Prüferinnen/Prüfern.
- Zusammensetzung und Arbeitsweise der Ethikkommission entsprechen nationalen Gesetzen, Vorschriften und der ICH-GCP-Leitlinie in der jeweils gültigen Fassung.
- Die Ethikkommission weist zusätzlich darauf hin, dass ihr Verdachtsfälle unerwarteter schwerwiegender Nebenwirkungen etc. unter Angabe und Zuordnung des Berichts nach § 42 Absatz 3 Satz 2 Nr. 1 AMG i.V.m. § 13 Absätze 2-5 GCP-V mitgeteilt werden müssen (siehe auch Anlage).

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IBAN DE89 3006 0601 0001 1452 90
BIC DAAEDEDD

Kernarbeitszeit: Montag bis Donnerstag 9 Uhr bis 16 Uhr, Freitag 9 Uhr bis 14 Uhr

004921143022279

ÄRZTEKAMMER NORDRHEIN
Körperschaft des öffentlichen Rechts

ETHIKKOMMISSION

Anhang 1 **lfd. Nummer 2014060**

Eingereichte Unterlagen: *Antrag eingegangen am 19.02.2014, Checkliste vom 13.01.2014, Email EudraCT vom 03.01.2014, Email Dr. Kroll vom 23.10.2013, Email Dr. Gabor vom 05.11.2013, Email Dr. Kroll vom 31.12.2013, Email Dr. Gabor vom 02.01.2014, Email Dr. Kroll vom 02.01.2014, Email Dr. Gabor vom 03.01.2014, Ausdruck Tiopronin aus orphanet, Prüfplan Version 12.01.2014, WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects, Anlage A4, Anlage A5a, Anlage A5b, Anlage A6, Fachinformation THIOLA®, Fachinformation Captimer Stand 2011, Anlage A8, Anlage A9, Anlage A10, Anlage A11, Anlage A12, Anlage A13, Anlage A14, Anlage A15, Erläuterung der Bedeutung der klinischen Prüfung, Anlage B2, Anlage B3, Anlage B4, Anlage B5, Aulistung der vom Sponsor übernommenen Kosten (in Euro), Lebenslauf Dr. med. Dr. rer. nat. Kroll vom 10.01.2014, Lebenslauf Dr. med. Pelzer vom 10.01.2014, Anlage B6a, Angaben zu wirtschaftlichen Interessen Dr. Kroll vom 03.01.2014, Angaben zu wirtschaftlichen Interessen Dr. Pelzer vom 03.01.2014, Anlage B8, Anlage B9, Anlage B10, Anlage B11, Anlage B12, Anlage B13, Angaben zur Vergütung Dr. Kroll und Dr. Pelzer vom 03.01.2014, Anlage B15, Vertrag zur Durchführung der klinischen Prüfung vom 13.01.2014, Anlage B17, Anlage B18, deutsche Zusammenfassung des Prüfplans, Modul 1 vom 13.01.2014, Modul 2 vom 12.01.2014, Angaben zum möglichen Einsatz einer Strahlenanwendung, Anlage 1 Modul 2, Anlage 2 Modul 2, Anlage 3 Modul 2, Anlage 4 Modul 2, Erklärung § 40 Abs. 1 Nr. 7 AMG Dr. Kroll vom 12.01.2014, Auflistung Primäres Zielkriterium und sekundäre Zielkriterien, Erklärung Ethische Überlegungen Dr. Kroll vom 03.01.2014, Anlage 7 Modul 2, Anlage 8 Modul 2, Anlage 9 Modul 2, Anlage 10 Modul 2, Anlage 11 Modul 2, Anlage 12 Modul 2, Anlage 13 Modul 2, Erklärung Einbeziehung abhängiger Personen Dr. Kroll vom 12.01.2014, Anlage 15 Modul 2, Anlage 16 Modul 2, Anlage 17 Modul 2, Bestätigung § 40 Abs. 2a AMG und § 7 Abs. 2 Nr. 15 GCP Dr. Kroll vom 12.01.2014, Anlage 19 Modul 2, Anlage 20 Modul 2, Anlage 21 Modul 2, Anlage 22 Modul 2, Anlage 23 Modul 2, AMG-Probanden-Information und –Einwilligung Version vom 10.01.2014, CRF, Beschreibung der Prüfgruppe unterzeichnet am 07.03.2014 und am 10.03.2014, Prüfstellenbeschreibung vom 07.03.2014, Beitragsangebot zur Probandenversicherung 40006501615-6 vom 07.02.2014, Allgemeine Versicherungsbedingungen AVBO01 Stand Februar 2014, Zertifikat GCP für Prüfärzte Dr. med. Dr. rer. nat. Kroll vom 06.03.2010, Fortbildungspunktekonto Dr. med. Dr. rer. nat. Kroll Stand 07.03.2014, Schreiben vom 16. Mai 2014 Nachforderung zu unserem Schreiben vom 12.03.2014 mit geänderter Patienteninformation, GCP-Nachweise von Herrn Dr. Dr. med. Michael Kroll und Herrn Dr. med. Michael Pelzer, Versicherungsunterlagen*

Ärztekammer Nordrhein
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ÄRZTEKAMMER NORDRHEIN
Körperschaft des öffentlichen Rechts

ETHIKKOMMISSION

Anhang 2 **lfd. Nummer 2014060**

Dr. med. Dr. rer. nat. Michael Kroll (Prüfer)
Dr. med. Michael Pelzer (Stellvertreter)
Berufsausübungsgemeinschat
Dr. med. Michael Pelzer
Dr. med. rer. nat. Michael Kroll
Hermannstr. 10
47533 Kleve

Ärztekammer Nordrhein
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ÄRZTEKAMMER NORDRHEIN
Körperschaft des öffentlichen Rechts

ETHIKKOMMISSION

Anhang 3

lfd. Nummer 2014060

Prof. Dr. med. Hermann Schulte-Wissermann
Arzt für Kinder- und Jugendmedizin
Vorsitzender des Gremiums III

Dr. med. Hans Uwe Feldmann
Arzt für Frauenheilkunde und Geburtshilfe
Arzt für Allgemeinmedizin
Person mit wissenschaftlicher oder beruflicher Erfahrung auf dem Gebiet der Ethik

Anke Franzen
Person aus dem Bereich der Patientenvertretung

Helmut Niedner
Rechtsanwalt
Vors. Richter am Verwaltungsgericht a.D.

Dr. med. Dr. jur. Frank Pluisch
Arzt für Rechtsmedizin

Dr. med. Brigitta Rumberger
Ärztin für Innere Medizin

Ulrike Schönau-Wendling
Apothekerin

Prof. Dr. med. Michael Zimmermann
Arzt für Neurochirurgie

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Kernarbeitszeit: Montag bis Donnerstag 9 Uhr bis 16 Uhr, Freitag 9 Uhr bis 14 Uhr

10.2 Subject Recruitment and Informed Consent

10.2.1 Recruitment poster

Probanden gesucht !

Getestet wird der Einfluss des Medikaments Captimer® auf den Abbau von Alkohol

Teilnahmevoraussetzung:

- Alter: 18 - 40 J.
- Geschlecht: ♂ oder ♀,
- Wenn ♀, keine bestehende, geplante Schwangerschaft
- Gesund, keine Vorerkrankungen oder derzeitige Tabletteneinnahme (Ausnahme : die Pille)

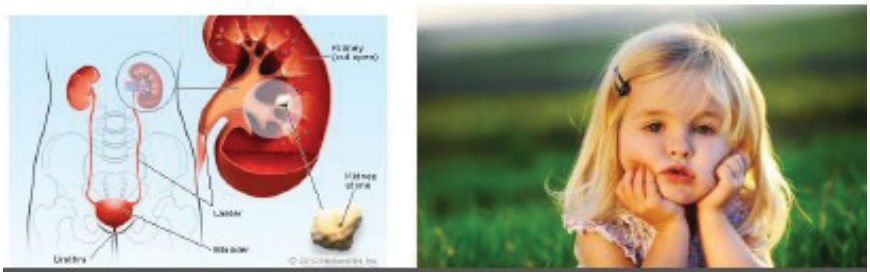
Teilnahmeinfo:

- Studiendauer: 1 Woche (zzgl. Vor- und Nachuntersuchung)
- Anzahl der Termine: 4
- Fixe Daten: 14.11.15 und 21.11.15
- Ort: Kleve (Niederrhein)
- Vergütung: EUR 300,-

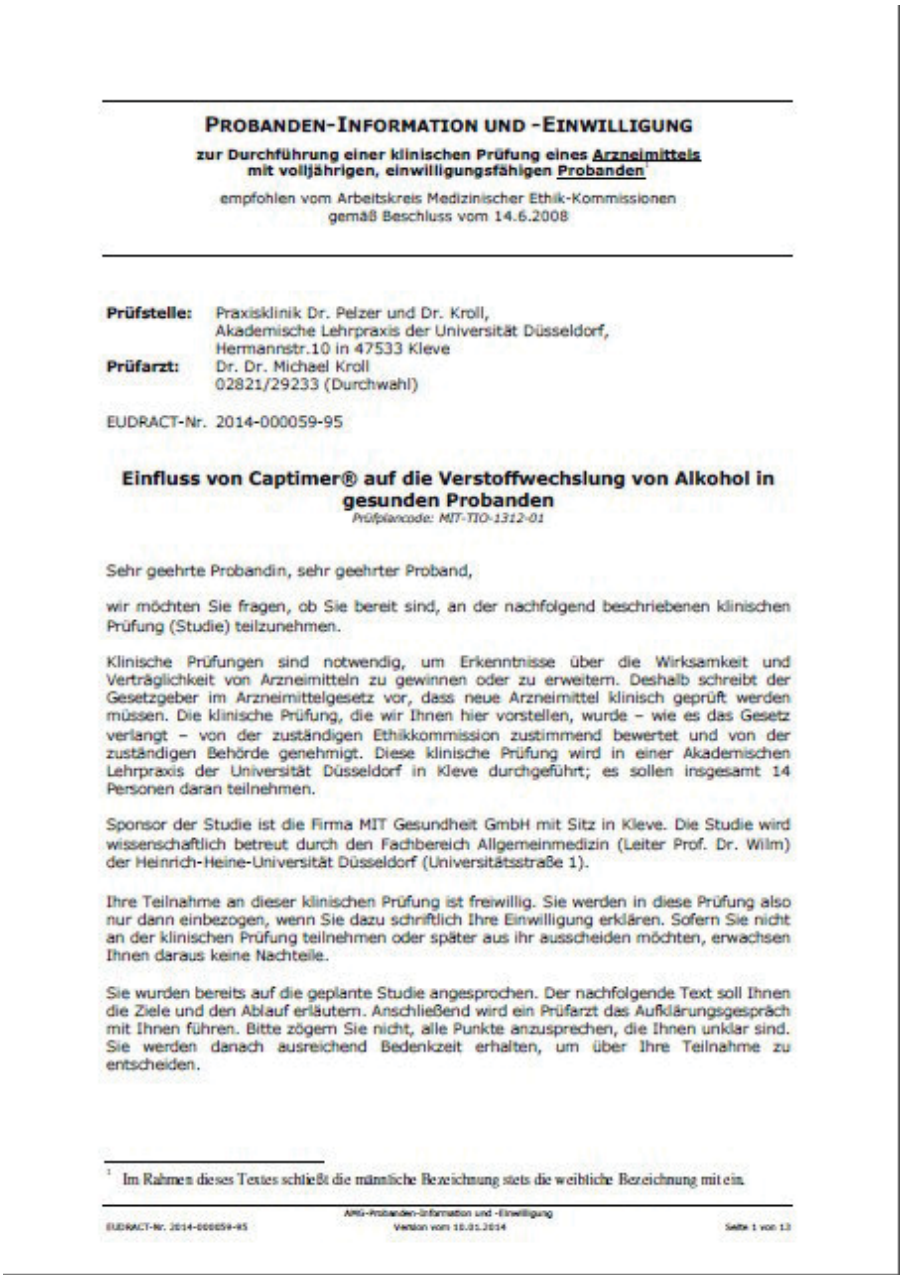
Captimer ®:

- Medikament seit 40 Jahren in Dtsl. zugelassen
- Hauptindikation Cystinurie (Stoffwechselerkrankung, bei der es schon im Kindesalter zu häufigen Nierensteinen kommt)

Kontakt: friederike.nass@t-online.de



10.2.2 Sample information and informed consent



PROBANDEN-INFORMATION UND -EINWILLIGUNG

zur Durchführung einer klinischen Prüfung eines Arzneimittels mit volljährigen, einwilligungsfähigen Probanden

empfohlen vom Arbeitskreis Medizinischer Ethik-Kommissionen
gemäß Beschluss vom 14.6.2008

Prüfstelle: Praxisklinik Dr. Pelzer und Dr. Kroll,
Akademische Lehrpraxis der Universität Düsseldorf,
Hermannstr.10 in 47533 Kleve
Prüfarzt: Dr. Dr. Michael Kroll
02821/29233 (Durchwahl)

EUDRACT-Nr. 2014-000059-95

Einfluss von Captimer® auf die Verstoffwechslung von Alkohol in gesunden Probanden
Prüfplancode: MIT-TIO-1312-01

Sehr geehrte Probandin, sehr geehrter Proband,

wir möchten Sie fragen, ob Sie bereit sind, an der nachfolgend beschriebenen klinischen Prüfung (Studie) teilzunehmen.

Klinische Prüfungen sind notwendig, um Erkenntnisse über die Wirksamkeit und Verträglichkeit von Arzneimitteln zu gewinnen oder zu erweitern. Deshalb schreibt der Gesetzgeber im Arzneimittelgesetz vor, dass neue Arzneimittel klinisch geprüft werden müssen. Die klinische Prüfung, die wir Ihnen hier vorstellen, wurde – wie es das Gesetz verlangt – von der zuständigen Ethikkommission zustimmend bewertet und von der zuständigen Behörde genehmigt. Diese klinische Prüfung wird in einer Akademischen Lehrpraxis der Universität Düsseldorf in Kleve durchgeführt; es sollen insgesamt 14 Personen daran teilnehmen.

Sponsor der Studie ist die Firma MIT Gesundheit GmbH mit Sitz in Kleve. Die Studie wird wissenschaftlich betreut durch den Fachbereich Allgemeinmedizin (Leiter Prof. Dr. Wilm) der Heinrich-Heine-Universität Düsseldorf (Universitätsstraße 1).

Ihre Teilnahme an dieser klinischen Prüfung ist freiwillig. Sie werden in diese Prüfung also nur dann einbezogen, wenn Sie dazu schriftlich Ihre Einwilligung erklären. Sofern Sie nicht an der klinischen Prüfung teilnehmen oder später aus ihr ausscheiden möchten, erwachsen Ihnen daraus keine Nachteile.

Sie wurden bereits auf die geplante Studie angesprochen. Der nachfolgende Text soll Ihnen die Ziele und den Ablauf erläutern. Anschließend wird ein Prüfarzt das Aufklärungsgespräch mit Ihnen führen. Bitte zögern Sie nicht, alle Punkte anzusprechen, die Ihnen unklar sind. Sie werden danach ausreichend Bedenkzeit erhalten, um über Ihre Teilnahme zu entscheiden.

[1] Im Rahmen dieses Textes schließt die männliche Bezeichnung stets die weibliche Bezeichnung mit ein.

1. Warum wird diese Prüfung durchgeführt?

Unsere Studie widmet sich dem bereits zugelassenen Medikament Captimer®. Dabei handelt es sich um ein sogenanntes Orphan Drug, ein Arzneimittel, welches zur Behandlung seltener Erkrankungen eingesetzt wird. Der in ihm enthaltene Wirkstoff heißt Tiopronin.

Captimer® ist ein in Deutschland zugelassenes Arzneimittel. Es werden derzeit ca. 400 Personen in Deutschland damit behandelt.

Hauptanwendungsgebiet von Captimer® sind Schwermetallvergiftungen und die Krankheit Zystinurie. Zystinurie ist eine erbliche Erkrankung, die mit einer vermehrten Ausscheidung von Zystin, einem Eiweißbaustein, einhergeht. Die Ausscheidung dieses Eiweißbausteins findet vor allem über die Nieren und dem dort gebildeten Harn statt. Eine erhöhte Konzentration von Zystin im Harn führt bei den erkrankten Personen oft zur Bildung von Zystinsteinen in den ableitenden Harnwegen. Das Medikament Captimer® ist in der Lage, die Löslichkeit von Zystin im Harn zu erhöhen und verhindert so die Bildung von Zystinsteinen bzw. kann diese wieder auflösen.

Die Probanden erkranken bereits im Kindesalter an Zystinurie und müssen dieses Medikament demnach ein Leben lang einnehmen. Die Lebenserwartung der Betroffenen hat sich glücklicher Weise in den letzten Jahren durch neu erforschte Therapie- und Behandlungsmöglichkeiten, zu denen auch Captimer® gehört, deutlich erhöht. Als Folge dessen ist auch der Zeitraum, in dem das Medikament eingenommen wird, deutlich angestiegen.

Während der Einnahme von Captimer® ist es den Probanden untersagt Alkohol zu trinken, da noch keine Studie am Menschen durchgeführt wurde, die eine mögliche Wechselwirkung zwischen Captimer® und Alkohol untersucht hat. Man weiß, dass das verwandte Medikament D-Penicillamin, welches auch zur Behandlung von Zystinurie eingesetzt wird und welches einen sehr ähnlichen chemischen Aufbau besitzt, keinen Einfluss auf die Verstoffwechslung von Alkohol hat. Von der hier vorgesehenen klinischen Prüfung erhoffen wir uns erste Erkenntnisse über die Wirkung von Captimer® auf den menschlichen Stoffwechsel von Alkohol.

2. Erhalte ich das Prüfpräparat auf jeden Fall?

Im Rahmen dieser klinischen Prüfung wird Captimer® mit einem Placebo verglichen. Bei einem Placebo handelt es sich um ein identisch aussehendes Arzneimittel, das jedoch keinen Wirkstoff enthält. Im Falle Ihrer Teilnahme werden Sie an einem Versuchstag Captimer® und an dem anderen Versuchstag das Placebo erhalten ("cross-over"). Der Vergleich mit dem Placebo dient dazu, die Wirkungen und Nebenwirkungen von Captimer® besser beurteilen zu können. Die Reihenfolge, in der dies geschieht, entscheidet ein zuvor festgelegtes Zufallsverfahren, vergleichbar mit dem Werfen einer Münze. Man nennt dies Randomisierung.

Zur objektiven Gewinnung von Studiendaten ist es notwendig, dass weder Sie noch Ihr Prüfarzt wissen, welches Medikament Sie an welchem Tag einnehmen (dieses Verfahren wird als "doppelblind" bezeichnet). Sollte es aus Sicherheitsgründen notwendig sein, kann unverzüglich festgestellt werden, welches Medikament Sie erhalten haben. Diese Studie wird demnach als randomisierte, doppelt-verblindete, Placebo-kontrollierte, cross-over Studie bezeichnet und gewährleistet damit den besten wissenschaftlichen Standart. Die Wahrscheinlichkeit, Captimer® an einem der beiden Versuchstage zu erhalten, beträgt 100 %.

3. Wie ist der Ablauf der Studie und was muss ich bei Teilnahme beachten?

Die Aufnahme in die klinische Prüfung erfolgt ca. einen Monat vor Studienbeginn. In diesem Zusammenhang erfolgt eine Anamnese (das ist ein systematisches Gespräch zur Erfassung aller medizinisch relevanten Daten) und Sie werden einer umfassenden ärztlichen Untersuchung unterzogen. Dazu gehört z.B. die Messung des Blutdrucks, des Pulses, des Gewichts und der Temperatur, das Abhören der Lunge und des Herzens, das Abtasten des Bauches, die Inspektion des Mundes und die Überprüfung des Pupillen- und des Patellarsehnenreflexes. Zudem werden wir Ihnen Blut abnehmen und Sie um eine Urinprobe bitten. Die Möglichkeit Ihrer weiteren Teilnahme an dieser klinischen Prüfung wird von den Ergebnissen dieser Voruntersuchung abhängen.

Sie werden an insgesamt 2 Wochenendtagen an der Studie teilnehmen. Sobald Sie in die Studie aufgenommen wurden, erhalten Sie einen Bescheid und erscheinen an den beiden Studientagen morgens nüchtern in der Praxis Hermannstraße 10, 47533 Kleve.

Jeweils 24 Stunden vor der Studie dürfen Sie keinen Alkohol trinken und nach 19:00 Uhr des Vortages keine Nahrung mehr zu sich nehmen. Nach einer kurzen Eingangsuntersuchung erhalten Sie von uns zu einem genau definierten Zeitpunkt das Placebo oder 500 mg Captimer®, welches sie dann mit 200 ml Wasser einnehmen. Genau eine Stunde später erhalten Sie die für Sie vorgesehene Menge Wodka (0,8 g Alkohol/kg Körpergewicht. Zur Geschmacksneutralisierung steht Ihnen während der Einnahme reichlich Wasser zur Verfügung. Zwischen der Einnahme des Medikaments und der Aufnahme von Alkohol, legen wir Ihnen einen venösen Zugang und nehmen die erste Blutprobe von 10 ml ab. Bei einem venösen Zugang handelt es sich um ein kleines Plastikröhrchen welches für die Dauer des Versuchstages in Ihrem Gefäß verweilt und über das das Prüfungsteam ohne einen weiteren Stich durch die Haut Blutabnehmen kann. Die Etablierung dieses Röhrchens erfolgt mit Hilfe einer Nadel, die in ihrer Dicke etwa den Nadeln der gewöhnlichen Blutabnahme entspricht.

In den folgenden 12 Stunden wird Ihnen in ebenfalls streng vorgegebenen Zeitabständen insgesamt zehn Mal ca. 10 ml Blut entnommen. Vor und nach dem Konsum des Alkohols werden jeweils noch ein Konzentrationstest (Zahlenverbindungstest) und ein Atemtest, welcher die Alkoholkonzentration in der Ausatmungsluft misst, vorgenommen. Es ist in dieser Studie wichtig die Zeitintervalle zwischen den Untersuchungen genau einzuhalten. Wir bitten Sie daher den Anweisungen des Prüfteams an den Versuchstagen exakt nachzugehen. Folgende Tabelle liefert Ihnen eine Übersicht über den Versuchsablauf.

Die Uhrzeiten sind vorläufige Annahmen und beziehen sich auf den ersten Probanden. Die Maßnahmen für die Behandlung der folgenden Probanden erfolgt zeitversetzt und kann sich unter Umständen verschieben.

	06:30	06:35	07:01	07:03	07:08	07:14	08:01	08:16	08:31	09:01	09:06	09:12	09:31	10:01	11:01	12:01	14:01	16:01	20:01	20:05
Ankunft	x																			
Gesundheits Check		x																		
Medikamenten/Placebo Einnahme			x																	
Venöser Zugang und Blutabnahme vor der Alkoholeinnahme				x																
ZVT1*[1]					x															
ABT1*[2]						x														
Einnahme von Alkohol							x													
Blutabnahme nach 1/4 h								x												
Blutabnahme nach 1/2 h									x											
Blutabnahme nach 1h										x										
ZVT2											x									
ABT2												x								
Blutabnahme nach 1 1/2 h													x							
Blutabnahme nach 2 h														x						
Blutabnahme nach 3 h															x					
Blutabnahme nach 4 h																x				
Blutabnahme nach 6 h																	x			
Blutabnahme nach 8 h																		x		
Blutabnahme nach 12 h																			x	
Abreise																				x

[1]Zahlenverbindungstest

[2]Atemalkoholmessung

	Vor-untersuchung	Studien-periode 1	Studien-periode 2	Nach-untesuchung
Anamnese	X			
Untersuchung	X	X	X	X
Blutabnahme für die Studie		X	X	
Blutdruck / Pulse	X	X	X	X
EKG	X			
Blutabnahme für ein Standardlabor	X			X
Schwangenschaftstest	X			

Nach Ablauf des Versuchstages können Sie das Praxisgelände verlassen. Es ist Ihnen jedoch untersagt, sich an diesem Tag im Straßenverkehr zu beteiligen.

Ca. 1 Woche später werden Sie den Studienablauf nochmals wiederholen, hierbei erhalten Sie dann das jeweils andere Präparat. Auch an diesem Tag ist es Ihnen untersagt, sich am Straßenverkehr zu beteiligen.

Die Nachuntersuchung findet in der Woche nach Ende der Studie, also nach Abschluss der zweiten Prüfperiode statt.

Sie dürfen im Zeitraum der Studie keine Medikamente (auch keine rezeptfreien) einnehmen oder sich einer anderen ärztlichen Behandlung (außer im Notfall) unterziehen. Ausnahmen sind hormonelle Kontrazeptiva (Anti-Baby-Pille). Sollten Sie nicht auf eine Medikation verzichten können, ist der Prüfarzt umgehend darüber zu informieren. Ihre Teilnahme an der Studie endet damit vorzeitig, ohne dass Ihnen ein Nachteil daraus erwächst. Über Ausnahmen entscheidet der Prüfarzt

Sie erhalten einen Studienausweis, den Sie auch für den Notfall immer mit sich führen sollten.

Alle Medikamente, die Sie im Verlauf dieser klinischen Prüfung bekommen, sollten Sie unverzüglich einnehmen. Die Abgabe an Dritte ist untersagt.

4. Welchen persönlichen Nutzen habe ich von der Teilnahme an der Studie?

Sie werden durch die Teilnahme an dieser Studie außer einer ärztlichen Untersuchung voraussichtlich keinen persönlichen Gesundheitsnutzen haben. Die Ergebnisse der Studie können aber möglicherweise dazu beitragen, die Behandlung der Zystinurie und von Schwermetallvergiftungen zukünftig zu verbessern/besser beurteilen zu können.

5. Welche Risiken sind mit der Teilnahme an der Studie verbunden?

Die Behandlung mit Captimer® kann zu unerwünschten Wirkungen oder Beschwerden führen. Die bislang beobachteten unerwünschten Wirkungen und Beschwerden umfassen:

Gelegentlich (0,1 - 1%) können auftreten:

- MagenDarmBeschwerden wie: Magenverstimmung, Durchfall (Diarrhöe)
- Störung des Geschmacksinns
- Temperaturerhöhung
- Hautreaktionen wie Juckreiz (Pruritus), Entzündung der Mundschleimhaut (Stomatitis),
- Blasenbildung (Pemphigus) sowie verschiedene Arten von Hautausschlägen (maculopapuläre Exantheme, Erythema multiforme und ekzematöse Eruptionen)

Selten (0,01 - 0,1%) wurde beobachtet:

- Anstieg der Leberwerte (Transaminasen), Leberentzündung (Hepatitis)
- Verminderung von Blutzellen (Thrombozytopenie, Leukopenie), in Einzelfällen kann eine Störung des Blutbildes eintreten (Agranulozytose)
- Nierenschädigung (Nephropathie)
- nephrotisches Syndrom: Krankheitsbild bei einer Nierenerkrankung mit großer Ausscheidung von Eiweißen im Urin (Proteinurie), Ansammlung von Wasser im Gewebe (Ödemen), erhöhten Blutfetten und Risiko zur Bildung eines Blutgerinnsels (Thrombose)
- Ausscheidung von Eiweißen im Urin (Albuminurie)
- Senkung des Blutzuckerspiegels.

Sehr selten (< 0,01%) wurde beobachtet:

- Erkrankung der Muskulatur (Myopathie)
- Erkrankung der Lunge (Pneumopathie)
- Entzündung der kleinen Luftwege (Bronchiolitis obliterans)
- krankhafte Muskelschwäche mit Lähmungserscheinungen (Myasthenia gravis)

Wie bei jedem Arzneimittel können auch bei der Anwendung von Captimer® neue, bisher unbekannte Nebenwirkungen auftreten.

Darüber hinaus können die im Rahmen dieser klinischen Prüfung studienbedingt durchgeführten Maßnahmen mit Risiken behaftet sein oder zu Beschwerden führen. Im Einzelnen handelt es sich um Risiken und Belastungen der Blutentnahme und um Risiken und Belastungen die durch das Trinken von Alkohol hervorgerufen werden können.

Bitte teilen Sie den Mitarbeitern der Prüfstelle *alle* Beschwerden, Erkrankungen oder Verletzungen mit, die im Verlauf der klinischen Prüfung auftreten. Falls diese schwerwiegend sind, teilen Sie den Mitarbeitern der Prüfstelle diese bitte umgehend mit, ggf. telefonisch.

Die Einnahme von Alkohol kann die Verkehrstüchtigkeit und das Bedienen von Maschinen beeinträchtigen. Sie dürfen deshalb an den beiden Versuchstagen nicht am Straßenverkehr teilnehmen.

6. Wer darf an dieser klinischen Prüfung nicht teilnehmen?

An dieser klinischen Prüfung dürfen Sie nicht teilnehmen, wenn Sie gleichzeitig an anderen klinischen Prüfungen oder anderen klinischen Forschungsprojekten teilnehmen oder vor kurzem teilgenommen haben.

Schwangere Frauen dürfen an dieser klinischen Prüfung **nicht teilnehmen**.

Zu Beginn der klinischen Prüfung und am Prüfungstag müssen sich deshalb alle Frauen einem Schwangerschaftstest unterziehen. Davon ausgenommen sind Frauen nach den Wechseljahren oder solche, die operativ sterilisiert wurden. Durch einen Schwangerschaftstest kann jedoch eine Schwangerschaft erst einige Tage nach der Empfängnis verlässlich nachgewiesen werden.

Im Falle Ihrer Teilnahme an dieser klinischen Prüfung müssen Sie zuverlässige Maßnahmen zur Schwangerschaftsverhütung anwenden und mit diesen vertraut sein. Dazu zählen hormonelle Kontrazeption (wie z.B. die Anti-Baby-Pille, hormonelle Depots wie Hormonspirale oder Verhütungspflaster), eine Spirale (IUP) oder sexuelle Enthaltsamkeit.

Der Grund dafür ist, dass bislang nicht geklärt ist, ob Captimer® zu einer Schädigung des Ungeborenen führen kann, wenn es während der Schwangerschaft eingenommen wird.

Sollten Sie während der klinischen Prüfung schwanger werden oder den Verdacht haben, dass Sie schwanger geworden sind, müssen Sie umgehend den Prüfarzt informieren.

Auch **stillende Frauen** dürfen an dieser klinischen Prüfung **nicht teilnehmen**, da Captimer® mit der Muttermilch in den Körper des Kindes gelangen und zu seiner Schädigung führen könnte.

7. Entstehen für mich Kosten durch die Teilnahme an der klinischen Prüfung? Erhalte ich eine Aufwandsentschädigung?

Durch Ihre Teilnahme an dieser klinischen Prüfung entstehen für Sie keine zusätzlichen Kosten.

Für Ihre Teilnahme an dieser klinischen Prüfung erhalten Sie eine Aufwandsentschädigung in Höhe von € 300.-- entsprechend den folgenden Bedingungen:

- Wenn Sie nur am ersten Studientag teilgenommen haben: gesamt € 100.--
- Wenn Sie an beiden Studientagen teilgenommen haben: gesamt € 300.--

Grundsätzliche Voraussetzung für eine Vergütung ist, dass Sie einer Abschlussuntersuchung unterziehen. Der Betrag wird an diesem Tage ausgehändigt.

Bitte beachten Sie, dass diese Vergütungen möglicherweise steuerpflichtig sind.

8. Bin ich während der klinischen Prüfung versichert?

Bei der klinischen Prüfung eines Arzneimittels sind alle Studienteilnehmer gemäß dem Arzneimittelgesetz versichert. Der Umfang des Versicherungsschutzes ergibt sich aus den Versicherungsunterlagen, die Sie in Kopie ausgehändigt bekommen.

Wenn Sie vermuten, dass durch die Teilnahme an der klinischen Prüfung Ihre Gesundheit geschädigt oder bestehende Leiden verstärkt wurden, müssen Sie dies unverzüglich dem Versicherer

Name und Anschrift der Versicherung:	Provinzial-Versicherung; Breite Straße 9, 47533 Kleve
Telefon:	02821/73310
Fax:	02821/733131
Versicherungsnummer:	40006501615-6

direkt anzeigen, gegebenenfalls mit Unterstützung durch Ihren Prüfarzt, um Ihren Versicherungsschutz nicht zu gefährden. Sofern Ihr Prüfarzt Sie dabei unterstützt, erhalten Sie eine Kopie der Meldung. Sofern Sie Ihre Anzeige direkt an den Versicherer richten, informieren Sie bitte zusätzlich Ihren Prüfarzt.

Bei der Aufklärung der Ursache oder des Umfangs eines Schadens müssen Sie mitwirken und alles unternehmen, um den Schaden abzuwenden und zu mindern.

Während der Dauer der klinischen Prüfung dürfen Sie sich einer anderen medizinischen Behandlung – außer in Notfällen – nur nach vorheriger Rücksprache mit dem Prüfarzt unterziehen. Von einer erfolgten Notfallbehandlung müssen Sie den Prüfarzt unverzüglich unterrichten.

Wir weisen Sie ferner darauf hin, dass Sie auf dem Weg von und zur Prüfstelle nicht unfallversichert sind.

9. Werden mir neue Erkenntnisse während der klinischen Prüfung mitgeteilt?

Sie werden über neue Erkenntnisse, die in Bezug auf diese klinische Prüfung bekannt werden und die für Ihre Bereitschaft zur weiteren Teilnahme wesentlich sein können, unverzüglich informiert. Auf dieser Basis können Sie dann Ihre Entscheidung zur weiteren Teilnahme an dieser klinischen Prüfung überdenken.

10. Wer entscheidet, ob ich aus der klinischen Prüfung ausscheide?

Sie können jederzeit, auch ohne Angabe von Gründen, Ihre Teilnahme beenden, ohne dass Ihnen dadurch irgendwelche Nachteile bei Ihrer medizinischen Behandlung entstehen.

Unter gewissen Umständen ist es aber auch möglich, dass der Prüfarzt oder der Sponsor entscheidet, Ihre Teilnahme an der klinischen Prüfung vorzeitig zu beenden, ohne dass Sie auf die Entscheidung Einfluss haben. Die Gründe hierfür können z.B. sein:

- Ihre weitere Teilnahme an der klinischen Prüfung ist ärztlich nicht mehr vertretbar;
- es wird die gesamte klinische Prüfung abgebrochen.

Sofern Sie sich dazu entschließen, vorzeitig aus der klinischen Prüfung auszuscheiden, oder Ihre Teilnahme aus einem anderen der genannten Gründe vorzeitig beendet wird, ist es für Ihre eigene Sicherheit und für den Versicherungsschutz wichtig, dass Sie sich einer empfohlenen abschließenden Kontrolluntersuchung unterziehen.

11. Welche Umstände können zu einem Abbruch der klinischen Prüfung führen?

Aufgrund der bisherigen Erkenntnisse ist nicht mit einem Abbruch der Studie zu rechnen. Umstände, die dennoch dazu führen könnten, sind neue Erkenntnisse über das Nutzen-Risiko-Verhältnisses des Medikaments und Abweichungen von dem im Prüfplan festgehaltenen Vorgehen (wie z.B. einer zu geringen Anzahl von Teilnehmern).

12. Was geschieht mit meinen Daten?

Während der klinischen Prüfung werden medizinische Befunde und persönliche Informationen von Ihnen erhoben und in der Prüfstelle in Ihrer persönlichen Akte niedergeschrieben und/oder elektronisch gespeichert. Die für die klinische Prüfung wichtigen Daten werden zusätzlich in pseudonymisierter Form gespeichert, ausgewertet und gegebenenfalls weitergegeben.

Pseudonymisiert bedeutet, dass keine Angaben von Namen oder Initialen verwendet werden, sondern nur ein Nummern- und/oder Buchstabencode, evtl. mit Angabe des Geburtsjahres.

Die Daten sind gegen unbefugten Zugriff gesichert. Eine Entschlüsselung erfolgt nur unter den vom Gesetz vorgeschriebenen Voraussetzungen oder in einem Notfall.

Das Arzneimittelgesetz enthält nähere Vorgaben für den erforderlichen Umfang der Einwilligung in die Datenerhebung und -verwendung. **Einzelheiten, insbesondere zur Möglichkeit eines Widerrufs, entnehmen Sie bitte der Einwilligungserklärung, die im Anschluss an diese Probandeninformation abgedruckt ist.**

13. Was geschieht mit meinen Blutproben?

Die Blutproben werden ausschließlich für diese klinische Prüfung verwendet. Die Laborwerte werden in anonymisierter Form in einem Datenprogramm erfasst. Etwaiges Restmaterial wird bei Abschluss der Prüfung vernichtet.

14. An wen wende ich mich bei weiteren Fragen?

Beratungsgespräche an der Prüfstelle

Sie haben stets die Gelegenheit zu weiteren Beratungsgesprächen mit dem auf Seite 1 genannten Prüfarzt.

Kontaktstelle

Es existiert außerdem eine Kontaktstelle bei der zuständigen Bundesoberbehörde. Teilnehmer an klinischen Prüfungen, ihre gesetzlichen Vertreter oder Bevollmächtigten können sich an diese Kontaktstelle wenden:

Bundesinstitut für Arzneimittel und Medizinprodukte
Fachgebiet Klinische Prüfung / Inspektionen
Kurt-Georg-Kiesinger-Allee 3
53175 Bonn

Telefon: 0228 / 207-4318 Fax: 0228 / 207-4355
e-mail: klinpruefung@bfarm.de

Prüfstelle: Gemeinschaftspraxis Dr. Pelzer und Dr. Dr. Kroll
Prüfarzt: Dr. Dr. Michael Kroll

EUDRACT-Nr. 2014-000059-95

Einfluss von Captimer® auf die Verstoffwechslung von Alkohol in gesunden Probanden
Prüfplancode MIT-TIO-1312-01

Einwilligungserklärung

.....
Name des Probanden in Druckbuchstaben

geb. am ..... Teilnehmer-Nr. .....

Ich bin in einem persönlichen Gespräch durch den Prüfarzt

.....
Name der Ärztin/des Arztes

ausführlich und verständlich über das Prüfmedikament sowie über Wesen, Bedeutung, Risiken und Tragweite der klinischen Prüfung aufgeklärt worden. Ich habe darüber hinaus den Text der Probandeninformation sowie die hier nachfolgend abgedruckte Datenschutzerklärung gelesen und verstanden. Ich hatte die Gelegenheit, mit dem Prüfarzt über die Durchführung der klinischen Prüfung zu sprechen. Alle meine Fragen wurden zufrieden stellend beantwortet.

Möglichkeit zur Dokumentation zusätzlicher Fragen seitens des Probanden oder sonstiger Aspekte des Aufklärungsgesprächs:

Ich hatte ausreichend Zeit, mich zu entscheiden.

Mir ist bekannt, dass ich jederzeit und ohne Angabe von Gründen meine Einwilligung zur Teilnahme an der Prüfung zurückziehen kann (mündlich oder schriftlich), ohne dass mir daraus Nachteile für meine medizinische Behandlung entstehen.

Datenschutz:

Mir ist bekannt, dass bei dieser klinischen Prüfung personenbezogene Daten, insbesondere medizinische Befunde über mich erhoben, gespeichert und ausgewertet werden sollen. Die Verwendung der Angaben über meine Gesundheit erfolgt nach gesetzlichen Bestimmungen und setzt vor der Teilnahme an der klinischen Prüfung folgende freiwillig abgegebene Einwilligungserklärung voraus, das heißt ohne die nachfolgende Einwilligung kann ich nicht an der klinischen Prüfung teilnehmen.

1. Ich erkläre mich damit einverstanden, dass im Rahmen dieser klinischen Prüfung personenbezogene Daten, insbesondere Angaben über meine Gesundheit, über mich erhoben und in Papierform sowie auf elektronischen Datenträgern bei/in der Gemeinschaftspraxis Dr. Pelzer und Dr. Dr. Kroll, 47533 Kleve (Lehrpraxis der Universität Düsseldorf) aufgezeichnet werden. Soweit erforderlich, dürfen die erhobenen Daten pseudonymisiert (verschlüsselt) weitergegeben werden:
   a) an den Zulassungsinhaber von Captimer (Firma MIT Gesundheit GmbH in Kleve), den Sponsor oder eine von diesem beauftragte Stelle zum Zwecke der wissenschaftlichen Auswertung (z.B. Statistiker),
   b) im Falle eines Antrags auf Zulassung oder Zulassungsänderung: an den Antragsteller und die für die Zulassung zuständige Behörde: Bundesinstitut für Arzneimittel und Medizinprodukte bzw. die Europäische Arzneimittelbehörde (EMA, European Medical Agency) oder andere Arzneimittelbehörden.
   c) im Falle unerwünschter Ereignisse: an den Zulassungsinhaber von Captimer (Firma MIT Gesundheit GmbH in Kleve), den Sponsor, an die jeweils zuständige Ethik-Kommission und die zuständige Bundesoberbehörde Bundesinstitut für Arzneimittel und Medizinprodukte, sowie von dieser an die Europäische Datenbank.

2. Außerdem erkläre ich mich damit einverstanden, dass autorisierte und zur Verschwiegenheit verpflichtete Beauftragte des Sponsors sowie die zuständigen Überwachungsbehörden in meine beim Prüfarzt vorhandenen personenbezogenen Daten, insbesondere meine Gesundheitsdaten, Einsicht nehmen, soweit dies für die Überprüfung der ordnungsgemäßen Durchführung der Studie notwendig ist. Für diese Maßnahme entbinde ich den Prüfarzt von der ärztlichen Schweigepflicht.

3. Die Einwilligung zur Erhebung und Verarbeitung meiner personenbezogenen Daten, insbesondere der Angaben über meine Gesundheit, ist unwiderruflich. Ich bin bereits darüber aufgeklärt worden, dass ich jederzeit die Teilnahme an der klinischen Prüfung beenden kann. Im Fall eines solchen Widerrufs meiner Einwilligung, an der Studie teilzunehmen, erkläre ich mich damit einverstanden, dass die bis zu diesem Zeitpunkt gespeicherten Daten weiterhin verwendet werden dürfen, soweit dies erforderlich ist, um
   a) Wirkungen des zu prüfenden Arzneimittels festzustellen,
   b) sicherzustellen, dass meine schutzwürdigen Interessen nicht beeinträchtigt werden,
   c) der Pflicht zur Vorlage vollständiger Zulassungsunterlagen zu genügen.

4. Ich erkläre mich damit einverstanden, dass meine Daten nach Beendigung oder Abbruch der Prüfung mindestens zehn Jahre aufbewahrt werden, wie es die Vorschriften über die klinische Prüfung von Arzneimitteln bestimmen. Danach werden meine personenbezogenen Daten gelöscht, soweit nicht gesetzliche, satzungsmäßige oder vertragliche Aufbewahrungsfristen entgegenstehen.

5. Ich bin über folgende gesetzliche Regelung informiert: Falls ich meine Einwilligung, an der Studie teilzunehmen, widerrufe, müssen alle Stellen, die meine personenbezogenen Daten, insbesondere Gesundheitsdaten, gespeichert haben, unverzüglich prüfen, inwieweit die gespeicherten Daten für die in Nr. 3 a) bis c) genannten Zwecke noch erforderlich sind.
   Nicht mehr benötigte Daten sind unverzüglich zu löschen.

6. Ich bin damit einverstanden, dass mein Hausarzt

   .....
   Name

   über meine Teilnahme an der klinischen Prüfung informiert wird.

Ich erkläre mich bereit,
an der oben genannten klinischen Prüfung
freiwillig teilzunehmen.

Ein Exemplar der Probanden-Information und -Einwilligung sowie die Versicherungsbedingungen habe ich erhalten. Ein Exemplar verbleibt im Prüfzentrum.

.....
Name des Probanden in Druckbuchstaben

....
Datum Unterschrift des **Probanden**

Ich habe das Aufklärungsgespräch geführt und die Einwilligung des Probanden eingeholt.

.....
Name des Prüfarztes/der Prüfärztin in Druckbuchstaben

....
Datum Unterschrift des aufklärenden **Prüfarztes/der Prüfärztin**

10.3 CASE REPORT FORM

Pre-Study-Visit (Set up)

	Pre-Study	Study Period 1	Study Period 2	Post-Study
Anamnesis	X			
Physical Examination	X	X	X	X
In-/Exclusion Criteria	X			
RR / Pulse	X	X	X	X
12 lead Standard ECG	X			
Concomitant Medication	X			
Routine laboratory tests	X			X
Urinalysis	X			X
Pregnancy Test (if applicable)	X			
Adverse Events		X	X	X
Application of Test Medication		X	X	
Test-Procedures		X	X	
Blood Drawings		X	X	

Pre-Study-Visit (Set up)

Inclusion Criteria

	yes	no
• Is this subject between 18 - 40 years old?		
• A female subject may be only enrolled if she is either a woman of non-childbearing potential* or a woman of childbearing potential, provided she	□	□
- has a negative pregnancy test before starting the study and - is routinely using adequate contraception (pill or IUP) prior to and during the study and - agrees not to become pregnant during the study	□	□
*A woman of non-childbearing potential is defined as - one who has been postmenopausal for at least 1 year or - was surgically sterilized or - had a hysterectomy at least three months prior to study start.		
• "healthy" as judged after a medical and laboratory examination by a physician	□	□
• Did the subject have signed a written informed consent form?	□	□

Only if all **inclusion criteria** were answered with "**yes**", the subject can be enrolled.

Pre-Study-Visit (Set-up)

Exclusion Criteria

	yes	no
• unable or unwilling to abide reliably by the requirements of the study protocol	☐	☐
• female subjects who are not using contraceptives, being not postmenopausal and being not surgically sterile, thus being of childbearing potential, but exhibiting a negative pregnancy test	☐	☐
• females who are pregnant or breast feeding	☐	☐
• hemodynamically relevant atrial or ventricular arrhythmia	☐	☐
• evidence of cerebrovascular accident within the last 6 months	☐	☐
• clinically significant pulmonary, hepatic, gastrointestinal, neurologic or haematological disease	☐	☐
• anamnestic suspected impairment of renal function, acute or chronic liver diseases, heart disease	☐	☐
• surgery or disease of the gastrointestinal tract or of other organs which in the opinion of the investigator might influence resorption or elimination of the drug	☐	☐
• acute autoimmune disease or clinically significant acute endocrine disease	☐	☐
• active wasting disease including cancer	☐	☐
• psychological and/or emotional problems which would render the informed consent invalid	☐	☐
• current or anamnestic - drug addiction, or - extensive chronic use of alcohol, or - history of alcoholism (so called "dry alcoholics")	☐	☐
• any co-medication (except oral anti-contraceptives in women)	☐	☐
• participation in another study with another investigational drug within 6 weeks prior to study enrolment	☐	☐
• limitation in exercise capacity (e.g. physical impairment)	☐	☐

➲ Only if all **exclusion criteria** were answered with "**no**", the subject can be enrolled.

Pre-Study-Visit (Set up)

Demographic Data

Date of Birth:_____/____/_____

Height: _____cm

Sex: ☐ male

☐ female

☐ oral contraception:_____ since:_____/____/_____

☐ hysterectomy on: ____/____/_____

☐ postmenopausal since:_____/____/_____

pregnancy test: ☐ negative ☐ positive; if positive -> EXCLUSION

Race: ☐ caucasian

☐ negroid

☐ asiatic

☐ oriental

☐ other, please specify: _____

Smoker: ☐ yes

☐ no

Pre-Study-Visit (set up)

Medical History

Diagnosis	daily dose	From (dd/mm/yy)	To (dd/mm/yy)	Ongoing
				□
				□
				□
				□
				□

Previous Surgical Procedures: □ **yes** □ **no**

If "yes", please document:

Diagnosis	Date (dd/mm/yy)

Did the subject receive any medication within the last three months? □ **yes** □ **no**

If "yes", please document:

Medication	daily dose	From (dd/mm/yy)	To (dd/mm/yy)	Ongoing
				□
				□
				□
				□

Pre-Study-Visit (Set up)

Physical Examination

Diagnosis	Findings normal*	Findings abnormal	If findings abnormal, please specify
Skin, Shape, Nodal lymphatic knots	□	□	
Heart,	□	□	
Lung	□	□	
Abdomen	□	□	
Neurological Tests	□	□	
Other findings (specify)	□	□	

*According Study Protocol

Pre-Study-Visit (Set up)

Vital functions

Diagnosis	Findings normal	Findings abnormal	Comments
Blood pressure (mmHg) _____/ _____ syst. / diast.	☐	☐	The subject must be excluded, if after 5 min at rest • SBP > 145 mmHg or DBP > 90 mmHg, or • SBP < 90 mmHg or DBP < 55 mmHg
Pulse rate (bpm) _____	☐	☐	Normal: 45 - 85/min., rhythmic
Temperature (°C): ___, __	☐	☐	Normal: 36°C - 37,5°C
BMI: ___	☐	☐	Normal: 19 - 28

ECG at rest (paper speed: 50 mm/sec, minimum of 3 cycles per lead))

Diagnosis	Findings normal	Findings abnormal	Comments, if clinically relevant
Heart position (Cabrera circle) _____	☐	☐	
PQ 0, ⌊_⌋⌊_⌋⌊_⌋ sec	☐	☐	Reference range: ≥ 0.08 sec and ≤ 0.20 sec
QT 0, ⌊_⌋⌊_⌋⌊_⌋ sec	☐	☐	Reference range: ≥ 0.25 sec and ≤ 0.45 sec
QRS 0, ⌊_⌋⌊_⌋⌊_⌋ sec	☐	☐	Reference range: ≥ 0.06 sec and ≤ 0.10 sec

Pre-Study-Visit (Set up)

ECG Tracing

Please attach ECG printout here.

Do not forget to enter

subject initials and date

on the tracking before signing it.

Pre-Study-Visit (Set up)

Laboratory(1/2)

Blood diagnostic	EDTA, Serum, Citrated blood			Value found	Comments
Haematology	Haemoglobin	m.: 13 - 17 g/dl	f.: 12 - 16 g/dl		
	Haematocrit	m.: 42 - 50%	f.: 38 - 44%		
	Erythrocytes	m.: 4,3 - 5,6 mill/µl	f.: 4,0 - 5,4 mill/µl		
	MCV MCH MCHC	85 - 98 fl 28 - 34 pg 31 - 37 g/dl			
	Thrombocytes	140 - 345 x1000/µl			
	Leucocytes	3800 - 9500 /µl			
Inflammation marker	CRP	≤ 5 mg/l			
	BSG	≤ 20 mm/h			
Coagulation laboratory	Quick	≥ 70%			
	INR	~1			

Pre-Study-Visit (Set up)

Laboratory(2/2)

Blood diagnostic	EDTA, Serum, Citrated blood			Value found	Comments
Organ specific parameter	γGT	m.: ≤ 60 U/l	f.: ≤ 40 U/l		
	GOT	m.: ≤ 38 U/l	f.: ≤ 34 U/l		
	GPT	m.: ≤ 41 U/l	f.: ≤ 31 U/l		
	Bilirubin total	≤ 1,1 mg/dl			
	CK	m.: ≤ 190 U/l	f.: ≤ 170		
	Creatinine	≤ 1,1 mg/dl			
	Urea	12 - 50 mg/dl			
Metabolic parameters	Uric acid	≤ 7 mg/dl			
	Glucose	≤ 100 mg/dl (fasting) or ≤ 130 mg/dl (after breakfast)			
Proteins	Proteins total	66 - 83 g/l			
Electrolytes	Sodium	135 - 145 mmol/l			
	Potassium	3,6 - 5,0 mmol/l			
	Calcium total	2,2 - 2,6 mmol/l			

Pre-Study-Visit (Set up)

Laboratory - Urinalysis

Parameter	Value		Comments
Pregnancy test (females only)	□ negative □ positive		
pH	___ □ normal □ abnormal		
Protein	□ negative (≤ 15mg/dl) □ 30 mg/dl □ 100 mg/dl □ 500 mg/dl □ not assessable		
Glucose	□ normal □ 50 mg/dl □ 100 mg/dl □ 300 mg/dl □ 500 mg/dl □ 1000 mg/dl		
Ketone bodies	□ negative □ + □ ++ □ +++		
Erythrocytes	□ negative □ 5 - 10 Ery/µl □ 50 Ery/µl □ 250 Ery/µl □ not assessable		
Nitrite	□ negative □ positive □ not assessable		
Bilirubin	□ negative □ + □ ++ □ +++ □ not assessable		
Urobilirubin	□ normal □ 1 mg/dl □ 4 mg/dl □ 8 mg/dl □ 12 mg/dl □ not assessable		
Leucocytes	□ negative □ 10 - 15 Leuco/µl □ 75 Leuco/µl □ ca 500 Leuco/µl □ not assessable		

Pre-Study-Visit (Set up)

After my carefully examination I confirm, that this subject is healthy and suitable to participate in the study.

_____	_____	_____
Place, Date	Medical Investigator	Signature

Study Period 1

	Pre-Study	Study Period 1	Study Period 2	Post-Study
Anamnesis	X			
Physical Examination	X	X	X	X
In-/Exclusion Criteria	X			
RR / Pulse	X	X	X	X
12 lead Standard ECG	X			
Concomitant Medication	X			
Routine laboratory tests	X			X
Urinalysis	X			X
Pregnancy Test (if applicable)	X			
Adverse Events		X	X	X
Application of Test Medication		X	X	
Test-Procedures		X	X	
Blood Drawings		X	X	

Study Period 1

Conditions

- Did the subject come to the clinic in fasting condition?
  □ yes
  □ no if "**no" ->** exclusion

- Did the subject consume alcohol in the last 24 hours?
  □ yes if "**yes**" -> exclusion
  □ no

Adverse events

During the last weeks since Pre-Study Visit,

- were there any new illness / abnormal finding / adverse event?
  □ yes ➲ if "**yes**" -> specify
  □ no

- did a concomitant illness / abnormal finding disappear?
  □ yes ➲ if "**yes**" -> specify
  □ no

Study Period 1

Physical Examination

Diagnosis	Findings normal*	Findings abnormal	If findings abnormal, please specify
Skin, Shape, Nodal lymphatic knots	□	□	
Heart,	□	□	
Lung	□	□	
Abdomen	□	□	
Neurological Tests	□	□	
Other findings (specify)	□	□	

*According Study Protocol

Study Period 1

Vital functions

Diagnosis	Findings normal	Findings abnormal	Comments
Blood pressure (mmHg) _____/ _____ syst. / diast.	☐	☐	The subject must be excluded, if after 5 min at rest • SBP > 145 mmHg or DBP > 90 mmHg, or • SBP < 90 mmHg or DBP < 55 mmHg
Pulse Rate (bpm) _____	☐	☐	Normal: 45 - 85/min., rhythmic

Study Period 1

After my carefully examination I confirm, that this subject is healthy and suitable to participate in the study.

_____	_____	_____
Place, Date	Medical Investigator	Signature

Study Period 1

Primary and Secondary Parameters

	Study Time	Time scheduled	Actual time	Signature
Application of test medication	**0**			
1st Blood drawing	**30 min after drug**			
Breath test	**35 min after drug**			
Number test	**35 min after drug**			
Application of alcohol	**1 hour after drug**			

Study Period 1

Primary and Secondary Parameters

2nd blood drawing	**0.25 h post alcoh.**			
3	**0.5 h post alcoh.**			
4	**1 h post alcoh.**			

Breath test	**1 hour post alcoh.**			
Number test	**1 hour post alcoh.**			

5	**1.5 h**			
6	**2 h**			
7	**3 h**			
8	**4 h**			
9	**6 h**			
10	**8 h**			
11	**12 h**			

Study-Period 2

	Pre-Study	Study Period 1	Study Period 2	Post-Study
Anamnesis	X			
Physical Examination	X	X	X	X
In-/Exclusion Criteria	X			
RR / Pulse	X	X	X	X
12 lead Standard ECG	X			
Concomitant Medication	X			
Routine laboratory tests	X			X
Urinalysis	X			X
Pregnancy Test (if applicable)	X			
Adverse Events		X	X	X
Application of Test Medication		X	X	
Test-Procedures		X	X	
Blood Drawings		X	X	

Study Period 2

Conditions

- Did the subject come to the clinic in fasting condition?
  - ☐ yes
  - ☐ no if "**no ->** exclusion
- Did the subject consume alcohol in the last 24 hours?
  - ☐ yes if "**yes**" -> exclusion
  - ☐ no

Adverse events

- were there any new illness / abnormal finding / adverse event?
  - ☐ yes ⇒ if "**yes**" -> specify
  - ☐ no
- did a concomitant illness / abnormal finding disappear?
  - ☐ yes ⇒ if "**yes**" -> specify
  - ☐ no

Study Period 2

Physical Examination

Diagnosis	Findings normal*	Findings abnormal	If findings abnormal, please specify
Skin, Shape, Nodal lymphatic knots	□	□	
Heart,	□	□	
Lung	□	□	
Abdomen	□	□	
Neurological Tests	□	□	
Other findings (specify)	□	□	

*According Study Protocol

Study Period 2

Vital functions

Diagnosis	Findings normal	Findings abnormal	Comments
Blood pressure (mmHg) _____/ _____ syst. / diast.	☐	☐	The subject must be excluded, if after 5 min at rest • SBP > 145 mmHg or DBP > 90 mmHg, or • SBP < 90 mmHg or DBP < 55 mmHg
Pulse Rate (bpm) _____	☐	☐	Normal: 45-85/min., rhythmic

Study Period 2

After my carefully examination I confirm, that this subject is healthy and suitable to participate in the study.

_____	_____	_____
Place, Date	Medical Investigator	Signature

Study Period 2

Primary and Secondary Parameters

	Study Time	Time scheduled	Actual time	Signature
Application of test medication	**0**			
1st Blood drawing	**30 min after drug**			
Breath test				
Number test	**30 min after drug**			
Application of alcohol	**1 hour after drug**			

Study Period 2

Primary and Secondary Parameters

2nd blood drawing	**0.25 h post alcohol**			
3	**0.5 h post alcohol**			
4	**1 h post alcohol**			

Breath test	**1 hour after alcohol**			
Number test	**1 hour after alcohol**			

5	**1.5 h**			
6	**2 h**			
7	**3 h**			
8	**4 h**			
9	**6 h**			
10	**8 h**			
11	**12 h**			

Post-Study-Visit

	Pre-Study	Study Period 1	Study Period 2	Post-Study
Anamnesis	X			
Physical Examination	X	X	X	X
In-/Exclusion Criteria	X			
RR / Pulse	X	X	X	X
12 lead Standard ECG	X			
Concomitant Medication	X			
Routine laboratory tests	X			X
Urinalysis	X			X
Pregnancy Test (if applicable)	X			
Adverse Events		X	X	X
Application of Test Medication		X	X	
Test-Procedures		X	X	
Blood Drawings		X	X	

Post-Study-Visit

Adverse events

During the last week since Study Period 2,

- were there any new illness / abnormal finding / adverse event?
  - ☐ yes ⇒ if "**yes**", -> follow up
  - ☐ no

- did a concomitant illness / abnormal finding disappear?
  - ☐ yes ⇒ if "**yes**" -> follow up
  - ☐ no

Post-Study-Visit

Physical Examination

Diagnosis	Findings normal*	Findings abnormal	If findings abnormal, please specify
Skin, Shape, Nodal lymphatic knots	□	□	
Heart,	□	□	
Lung	□	□	
Abdomen	□	□	
Neurological Tests	□	□	
Other findings (specify)	□	□	

*According Study Protocol

Post-Study-Visit

Vital functions

Diagnosis	Findings normal	Findings abnormal	Comments
Blood pressure (mmHg) _____/ _____ syst. / diast.	☐	☐	The subject must be excluded, if after 5 min at rest • SBP > 145 mmHg or DBP > 90 mmHg, or • SBP < 90 mmHg or DBP < 55 mmHg
Pulse rate (bpm) _____	☐	☐	Normal: 45-85/min., rhythmic

Post-Study-Visit

Laboratory

Blood diagnostic	EDTA, Serum, Citrated blood			Value found	Comments
Haematology	Haemoglobin	m.: 13 - 17 g/dl	f.: 12 - 16 g/dl		
	Haematocrit	m.: 42 - 50%	f.: 38 - 44%		
	Erythrocytes	m.: 4,3 - 5,6 mill/µl	f.: 4,0 - 5,4 mill/µl		
	MCV MCH MCHC	85 - 98 fl 28 - 34 pg 31 - 37 g/dl			
	Thrombocytes	140 - 345 x1000/µl			
	Leucocytes	3800 - 9500 /µl			
Coagulation laboratory	Quick	≥ 70%			
	INR	~1			
Organ specific parameter	γGT	m.: ≤ 60 U/l	f.: ≤ 40 U/l		
	GOT	m.: ≤ 38 U/l	f.: ≤ 34 U/l		
	GPT	m.: ≤ 41 U/l	f.: ≤ 31 U/l		
	Bilirubin total	≤ 1,1 mg/dl			
	CK	M.: ≤ 190 U/l	f.: ≤ 170		
	Creatinine	≤ 1,1 mg/dl			
	Urea	12 - 50 mg/dl			
Metabolic parameters	Uric acid	≤ 7 mg/dl			
Proteins	Proteins total	66 - 83 g/l			

Post-Study-Visit

Laboratory - Urinalysis

Parameter	Value		Comments
pH	___ □ normal □ abnormal		
Protein	□ negative (≤15mg/dl) □ 30 mg/dl □ 100 mg/dl □ 500 mg/dl □ not assessable		
Glucose	□ normal □ 50 mg/dl □ 100 mg/dl □ 300 mg/dl □ 500 mg/dl □ 1000 mg/dl		
Ketone bodies	□ negative □ + □ ++ □ +++		
Erythrocytes	□ negative □ 5 - 10 Ery/µl □ 50 Ery/µl □ 250 Ery/µl □ not assessable		
Nitrite	□ negative □ positive □ not assessable		
Bilirubin	□ negative □ + □ ++ □ +++ □ not assessable		
Urobilirubin	□ normal □ 1 mg/dl □ 4 mg/dl □ 8 mg/dl □ 12 mg/dl □ not assessable		
Leucocytes	□ negative □ 10 - 15 Leuco/µl □ 75 Leuco/µl □ ca 500 Leuco/µl □ not assessable		

Post-Study-Visit

After my carefully examination I confirm that this subject completed the study according to protocol and is healthy as judged after my examination.

Place, Date	Medical Investigator	Signature

Final Assessment

Study end regular ☐

premature ☐

Dates

End of study: ⌊_⌊_⌋ / ⌊_⌊_⌋ / ⌊_⌊_⌋

dd mm yy

In case of premature study end

The subject discontinued due to

☐ Adverse event go to page AE or SAE for documentation

☐ Occurrence of exclusion criteria specify: _____

☐ Protocol violation

☐ Non-compliance

☐ Subject's request

☐ Withdrawal of informed consent

☐ Other reason specify: _____

Emergency envelope

Emergency envelope opened	☐ yes	date: ⌊_⌊_⌋ / ⌊_⌊_⌋ / ⌊_⌊_⌋ specify: _____
	☐ no	
	☐ not applicable	

Adverse Events

	Adverse Event (per line only one single event!)	Serious	Start End	Intensity	Relationship to study drug	Actions taken	Outcome
1		☐ yes ☐ no	¹–†–¹/¹–†–¹/¹–†–¹ dd / mm / yy ¹–†–¹/¹–†–¹/¹–†–¹ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ unknown ☐ not related	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
2		☐ yes ☐ no	¹–†–¹/¹–†–¹/¹–†–¹ dd / mm / yy ¹–†–¹/¹–†–¹/¹–†–¹ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
3		☐ yes ☐ no	¹–†–¹/¹–†–¹/¹–†–¹ dd / mm / yy ¹–†–¹/¹–†–¹/¹–†–¹ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
4		☐ yes ☐ no	¹–†–¹/¹–†–¹/¹–†–¹ dd / mm / yy ¹–†–¹/¹–†–¹/¹–†–¹ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown

SAE

Serious Adverse Event Emergency Report Form

Protocol No.: **Report type:** ☐ initial ☐ follow up

Subject Data:

Sex: ☐ male ☐ female

Date of birth ⌊⌊ / ⌊⌊ / ⌊⌊
dd / mm / yy

Adverse Event	Reason for seriousness	Start End	Last application of study drug	Relationship to study drug	Actions taken	Outcome
	☐ fatal ☐ life-threatening ☐ disabling ☐ (prolonged) hospitali-zation ☐ congenital anomaly ☐ malignancy	⌊⌊/⌊⌊/⌊⌊ dd / mm / yy ⌊⌊/⌊⌊ hh / min ⌊⌊/⌊⌊/⌊⌊ dd / mm / yy ⌊⌊/⌊⌊ hh / min	⌊⌊/⌊⌊/⌊⌊ dd / mm / yy ⌊⌊/⌊⌊ hh / min	☐ definite ☐ probable ☐ possible ☐ unknown ☐ not related	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown

During which period ☐ wash out ☐ run-in Study drug dose: _____mg / day

did the event appear? ☐ treatment ☐ follow-up Treatment unblinded ☐ yes ☐ no

Further details of event(s): _____

_____

Relevant tests / lab. data: _____

_____

Risk factors _____

_____

Concomitant diseases: _____

_____

SAE

Concomitant drugs

No	Medication (trade name)	Daily dose (mg)	Start (dd/mm/yy)	End (dd/mm/yy)	Ongoing
1			⌊⌊/⌊⌊/⌊⌊ dd / mm / yy	⌊⌊/⌊⌊/⌊⌊ dd / mm / yy	☐ yes
2			⌊⌊/⌊⌊/⌊⌊ dd / mm / yy	⌊⌊/⌊⌊/⌊⌊ dd / mm / yy	☐ yes
3			⌊⌊/⌊⌊/⌊⌊ dd / mm / yy	⌊⌊/⌊⌊/⌊⌊ dd / mm / yy	☐ yes
4			⌊⌊/⌊⌊/⌊⌊ dd / mm / yy	⌊⌊/⌊⌊/⌊⌊ dd / mm / yy	☐ yes
5			⌊⌊/⌊⌊/⌊⌊ dd / mm / yy	⌊⌊/⌊⌊/⌊⌊ dd / mm / yy	☐ yes

In case of death:

Primary cause of death: _____

(if possible, add report of hospital or autopsy)

_____

Possible explanation for SAE:

☐ underlying disease ☐ intercurrent disease ☐ drug interaction

☐ concomitant disease ☐ concomitant drug ☐ study drug

Relationship of SAE to study drug:

(Please comment:) _____

_____

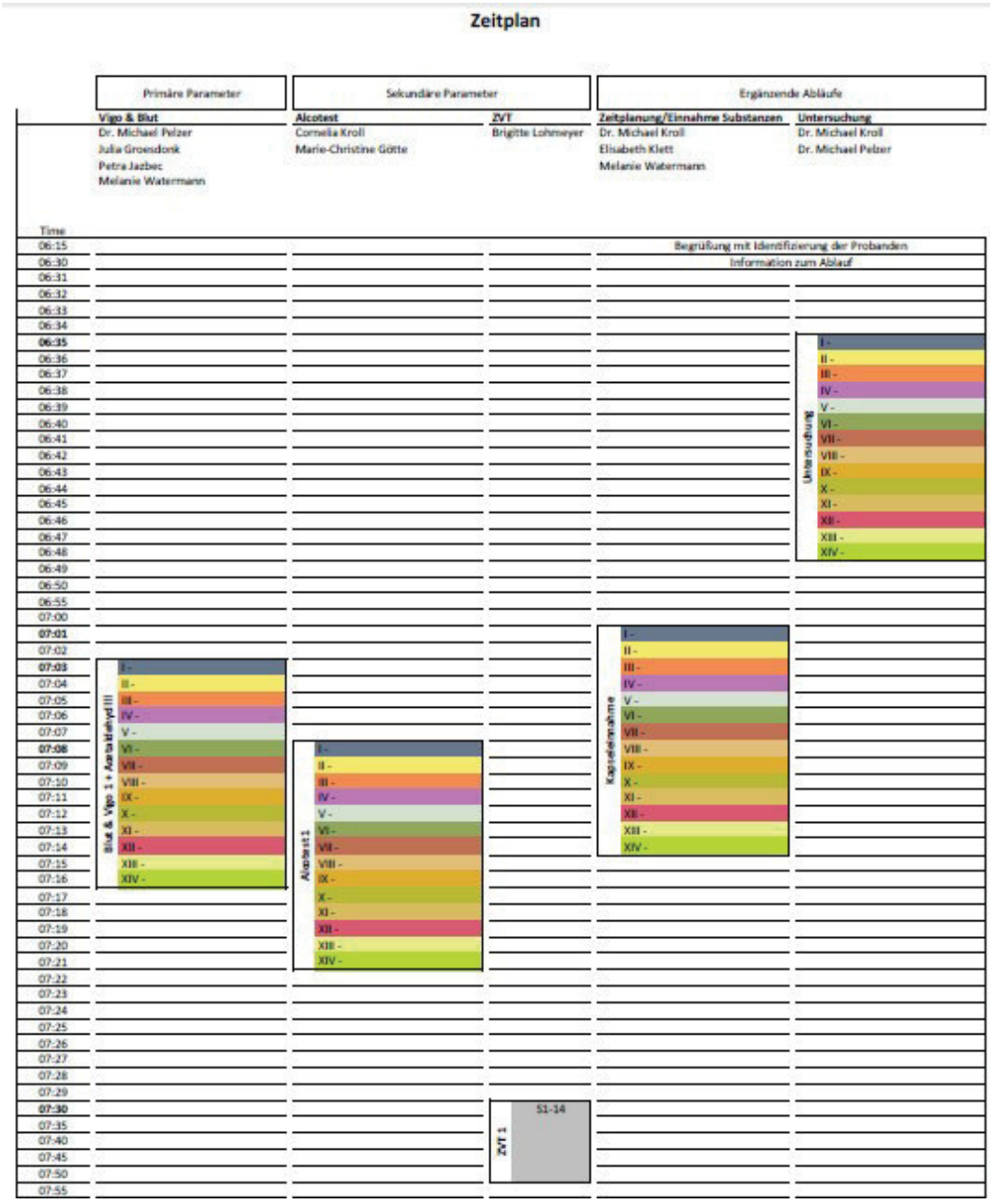
Investigator:

Name of reporter (block letters): Phone:_____ Date: _____

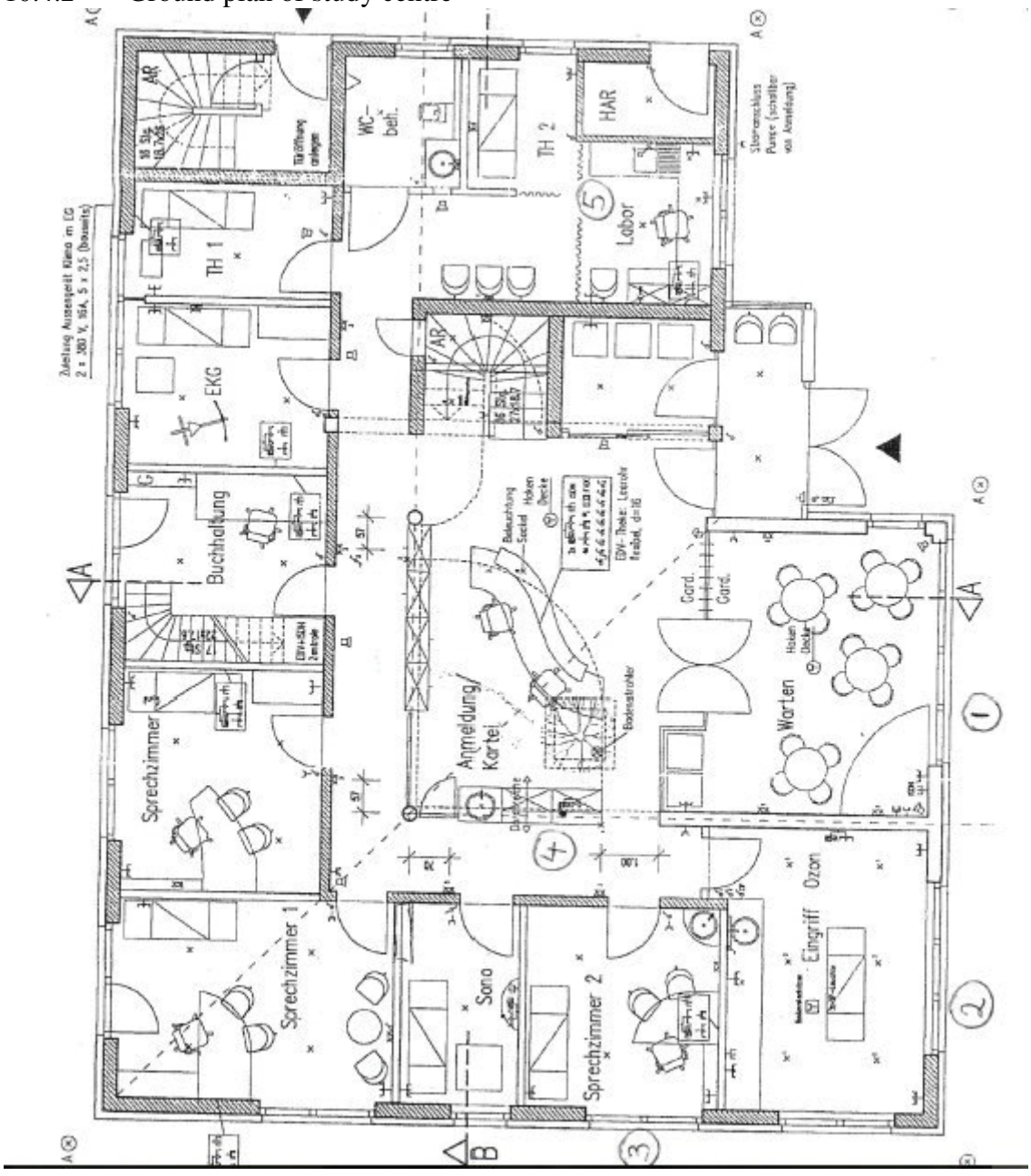
_____ Fax: _____ Signature: _____

10.4 CONDITIONS OF THE STUDY

10.4.1 Sample of time schedule



10.4.2 Ground plan of study centre



1: waiting room:	- administration of study drug - administration of alcohol - ZVT
2: surgery ward:	- blood draws - venous accesses
3: consulting room:	- clinical examination - vital parameters - AE observation and treatment
4: passage	- Alcotest 6810
5: laboratory:	- centrifuging - pipetting

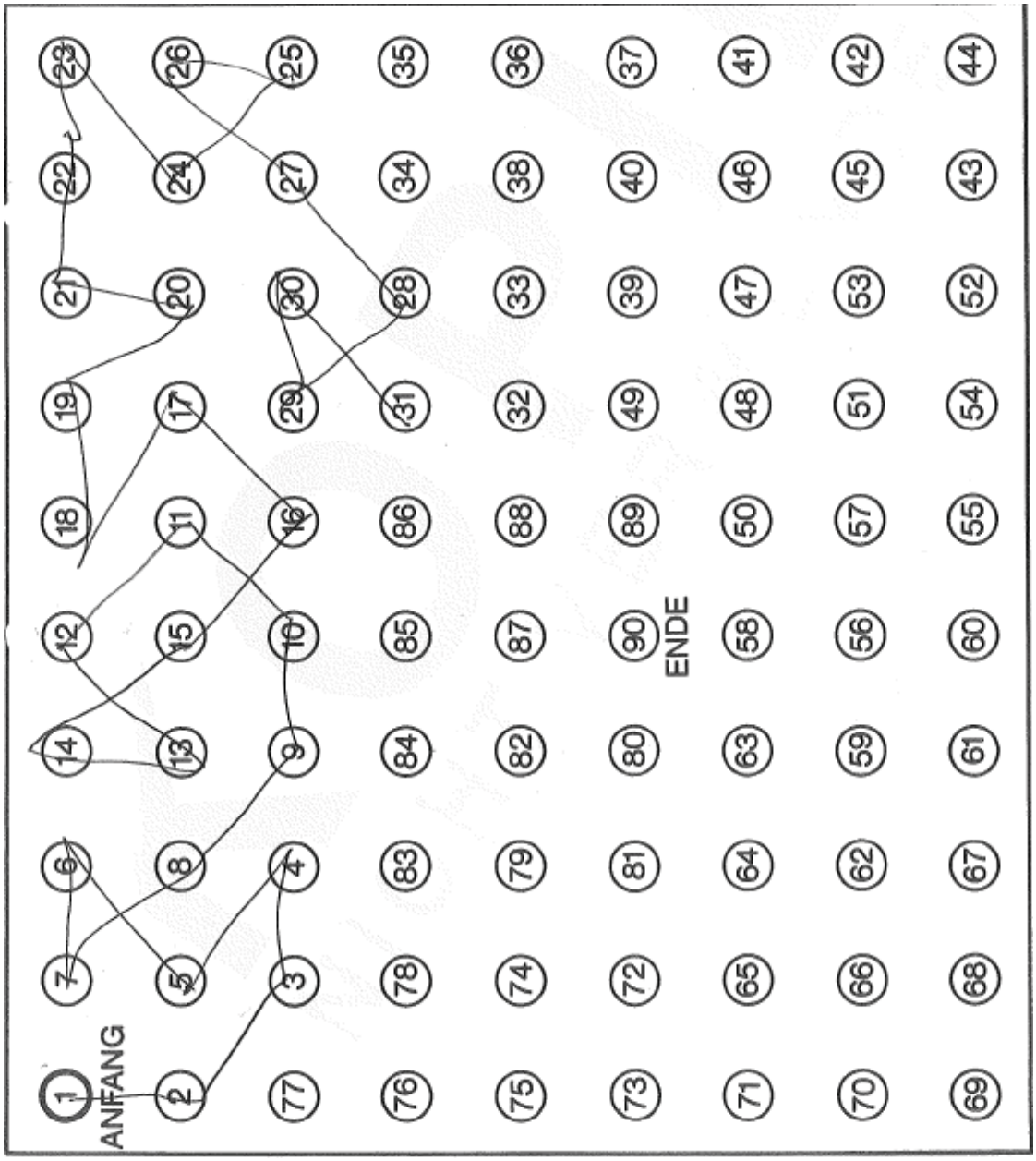
Table 18: Functions of individual areas

10.4.3 Study team members

NAME	ROLE	QUALIFICATION
Dr. J.K. Merges	Sponsor	CEO MIT Gesundheit GmbH
Dr. E. Gabor	Trial director	Head of Pharmacovigilance Department
Dr. Dr. M. Kroll	Principle Investigator	Qualifications: Graduate Plan Manager (1990), Principal Investigator (1991), Information Officer(1996), Quality Manager(1997), Auditor(1997), Assessor(1998), Medical Quality Manager(1998), Doctor for General Practice (2002) and Palliative Medicine (2012)
Dr. M. Pelzer	Representative Principle Investigator	Qualifications: Doctor for General Practice (1998), Emergency Doctor(2007), Doctor for Palliative Medicine, Teaching Position Emergency Service (2008), Teaching Position University Rhine-Waal (2009), Co-operation in Different Clinical Trials
Prof. S. Wilm	Pharmacovigilance Medical Advisor	Head of Institute of General Practice HHU Duesseldorf (http://www.uniklinik-duesseldorf.de/allgemeinmedizin)
Prof. B. Schneider	Statistician	Emeritus Institute for Biometrics MH Hannover (https://www.mh-hannover.de/berthold_schneider.html)
E. Klett	Study Assistant	Medical assistant experienced and skilled in clinical trails
F. Naß	Monitor, Author of Study Report	Medical student, advanced training for clinical trials (KKS Duesseldorf 2013),
P. Jazbec	Study Assistant	Examined practice nurse, skilled in clinical trials
M. Watermann	Study Assistant	Examined practice nurse, skilled in clinical trials
J. Groesdonk	Study Assistant	Examined practice nurse, skilled in clinical trials
B. Lohmeyer	Study Assistant	Examined practice nurse, skilled in clinical trials
M.C. Götte	Study Assistant	Medical student, skilled in clinical trials
C. Kroll	Study Assistant	Lawyer, experienced and skilled in clinical trials
M. Naß	Study Assistant	Examined medical technical assistant, skilled in clinical trials
B. Kardel	Responsible Person Laboratory Duesseldorf	Dipl. Ing. Forensic Toxicology University of Duesseldorf
A. Abraham	Responsible Person Laboratory Goch	Principle medical technical assistant Central Laboratory Goch

10.5 ADDITIONAL MATERIALS

Sample Matrix ZVT



10.6 ADDITIONAL RESULTS

10.6.1 Individual alcohol time-concentration profiles

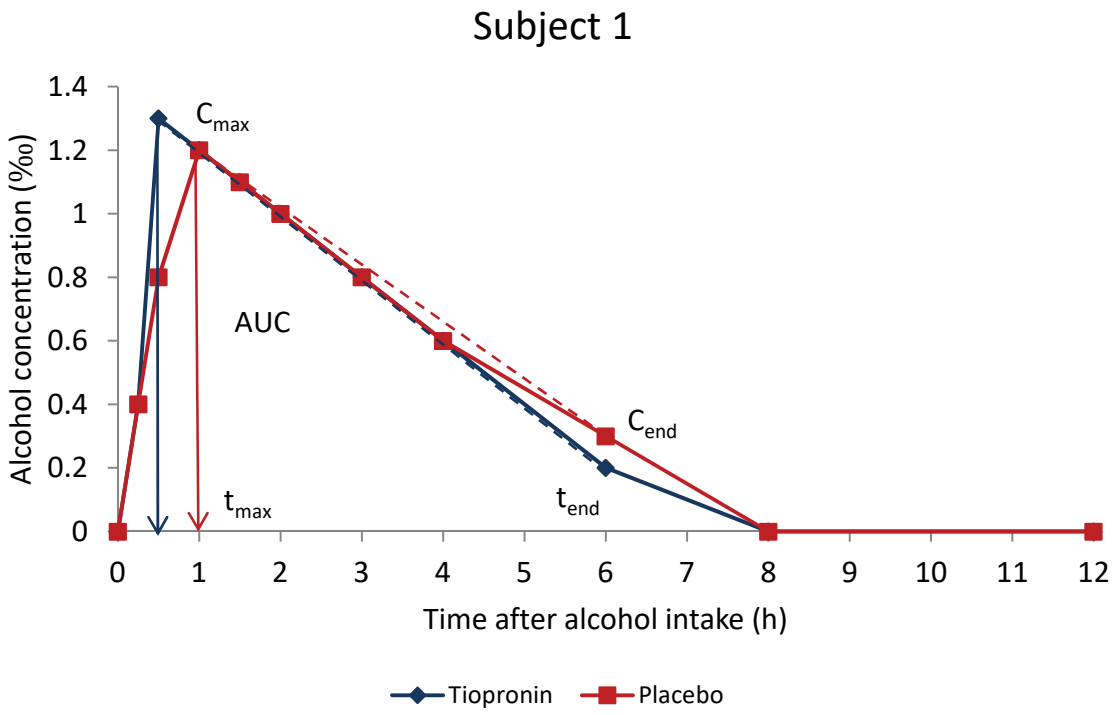


Fig. 8: Concentration-time profile subject 1

Subject 1	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Tiopronin	0.5	1.3	4.59	0.20
Placebo	1.0	1.2	4.60	0.18

Table 1: Kinetic parameters subject 1

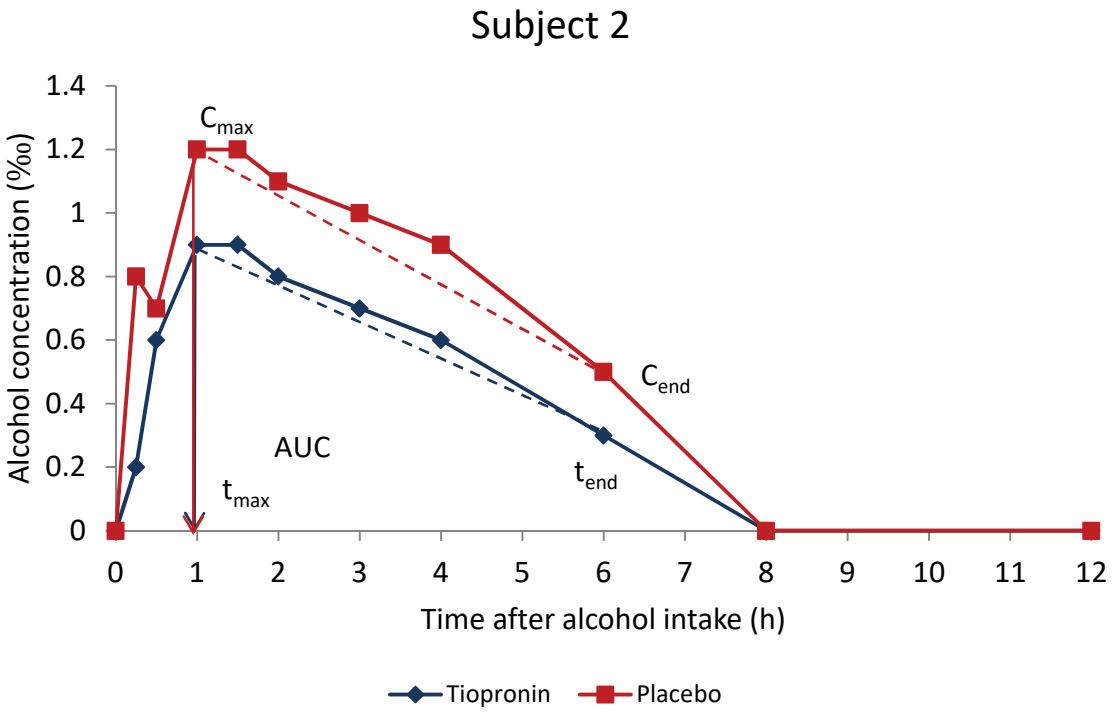


Fig. 9: Concentration-time profile subject 2

Subject 2	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Tiopronin	1.0	0.9	3.97	0.13
Placebo	1.0	1.2	5.84	0.16

Table 2: Kinetic parameters subject 2

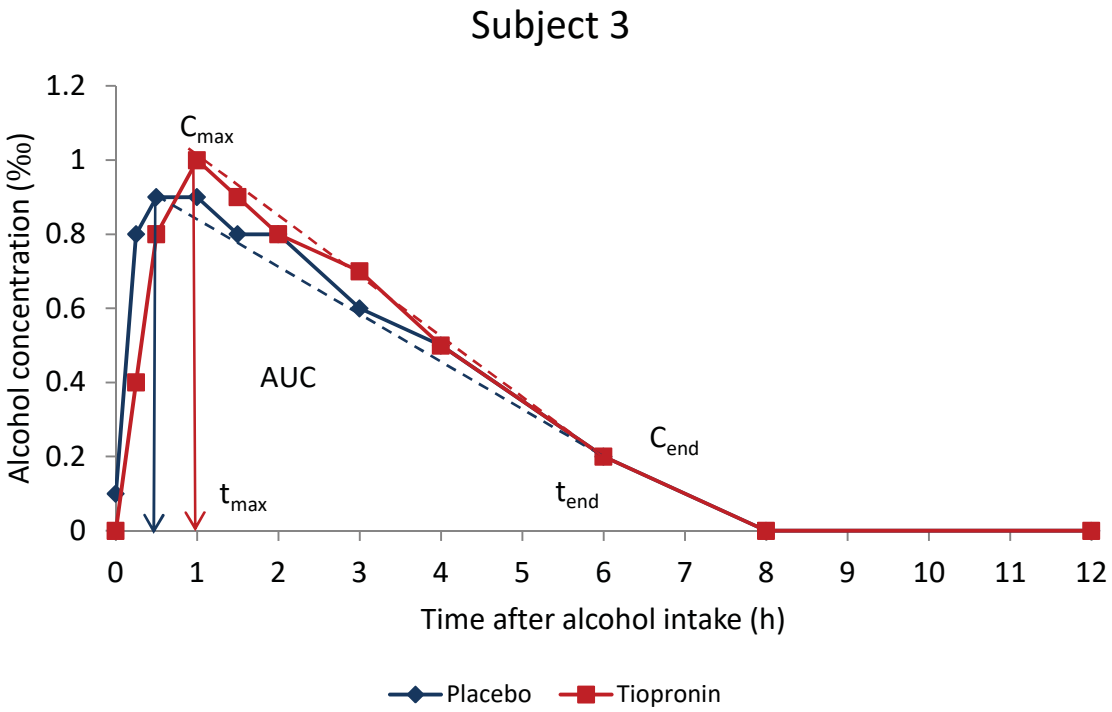


Fig. 10: Concentration-time profile subject 3

Subject 3	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Placebo	0.5	0.9	3.75	0.14
Tiopronin	1.0	1.0	3.80	0.16

Table 3: Kinetic parameters subject 3

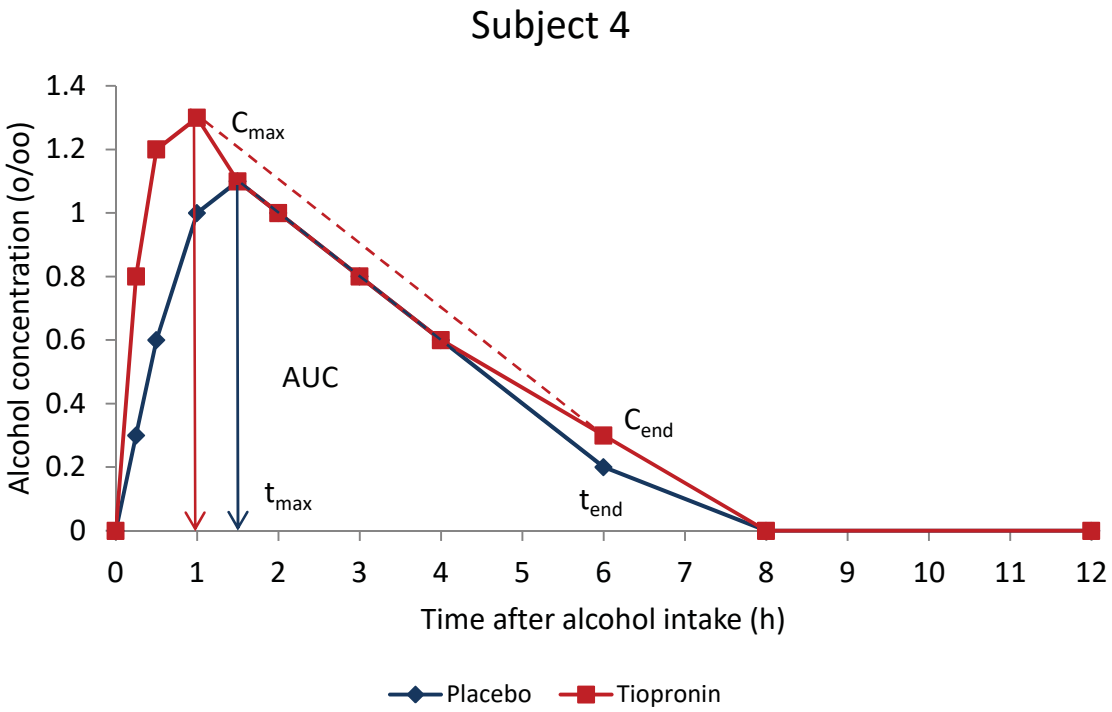


Fig. 11: Concentration-time profile subject 4

Subject 4	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Placebo	1.5	1.1	4.2	0.20
Tiopronin	1.0	1.3	4.9	0.20

Table 4: Kinetic parameters subject 4

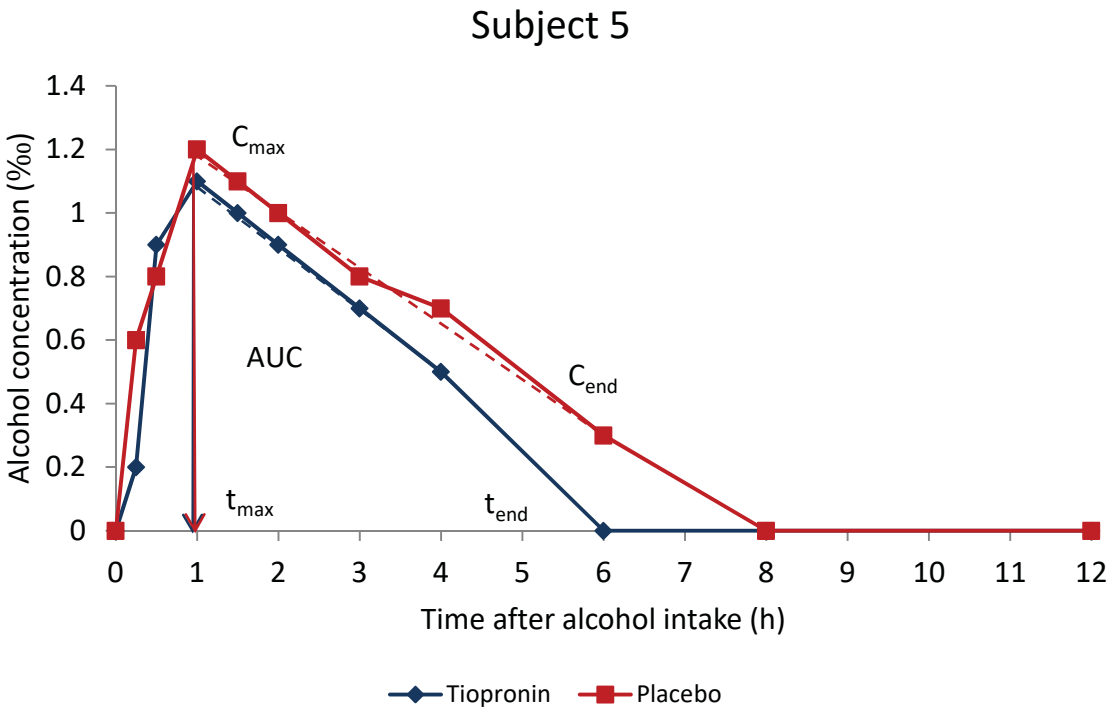


Fig. 12: Concentration-time profile subject 5

Subject 5	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Tiopronin	1.0	1.1	3.56	0.20
Placebo	1.0	1.2	4.81	0.18

Table 5: Kinetic parameters subject 5

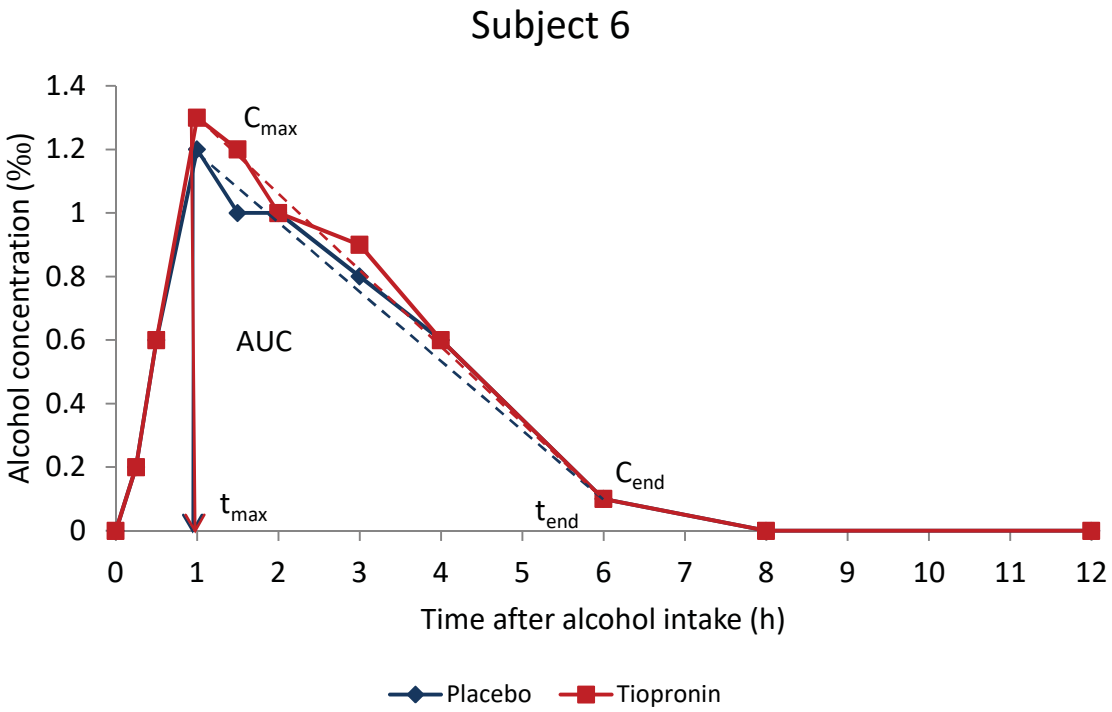


Fig. 13: Time-concentration profile subject 6

Subject 6	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Placebo	1.0	1.2	4.02	0.22
Tiopronin	1.0	1.3	4.27	0.24

Table 6: Kinetic parameters subject 6

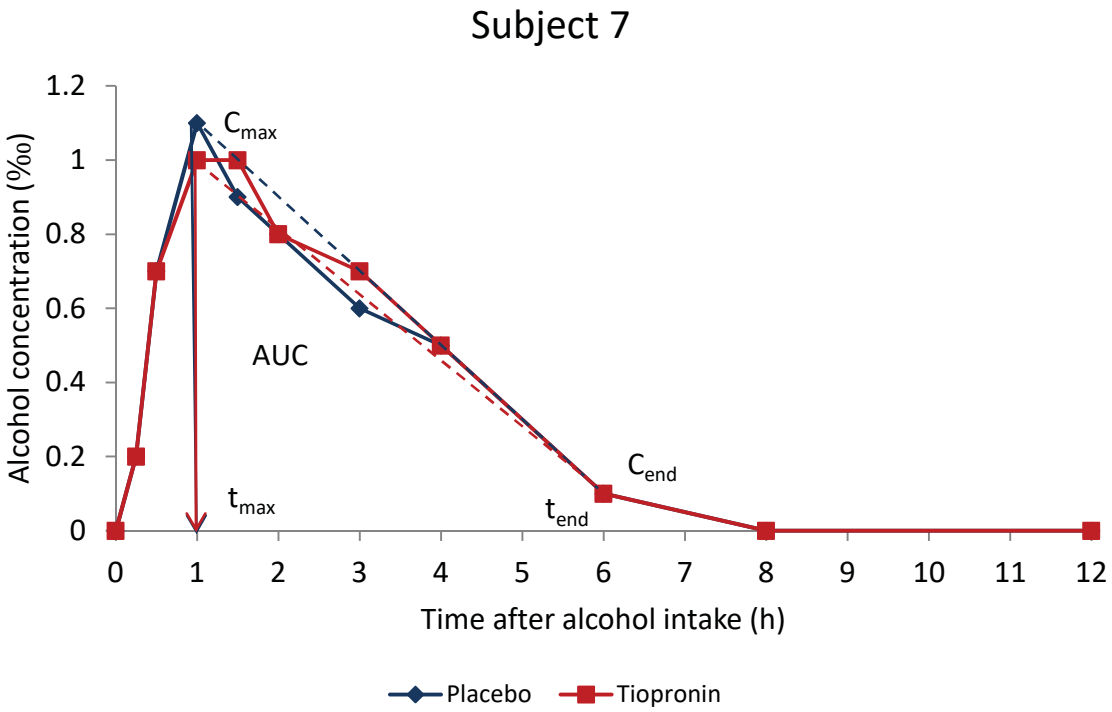


Fig. 14: Concentration-time profile subject 7

Subject 7	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Placebo	1.0	1.1	3.46	0.20
Tiopronin	1.0	1.0	4.56	0.20

Table 7: Kinetic parameters subject 7

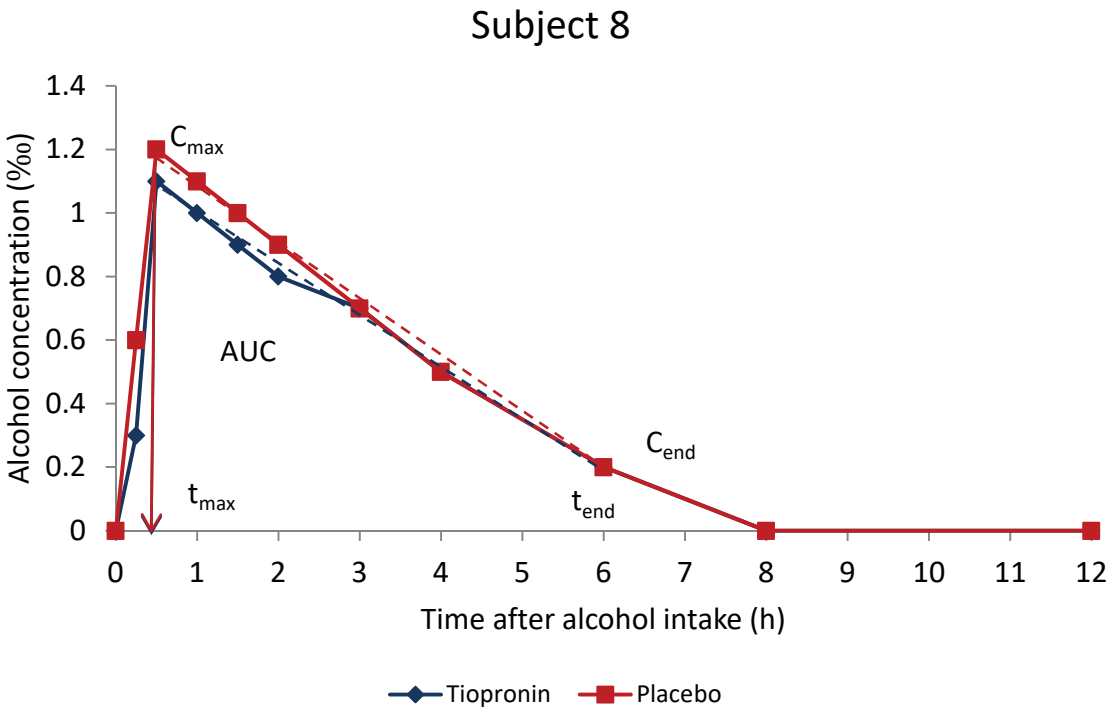


Fig. 15: Concentration-time profile subject 8

Subject 8	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Tiopronin	0.5	1.1	3.89	0.16
Placebo	0.5	1.2	4.18	0.18

Table 8: Kinetic parameters subject 8

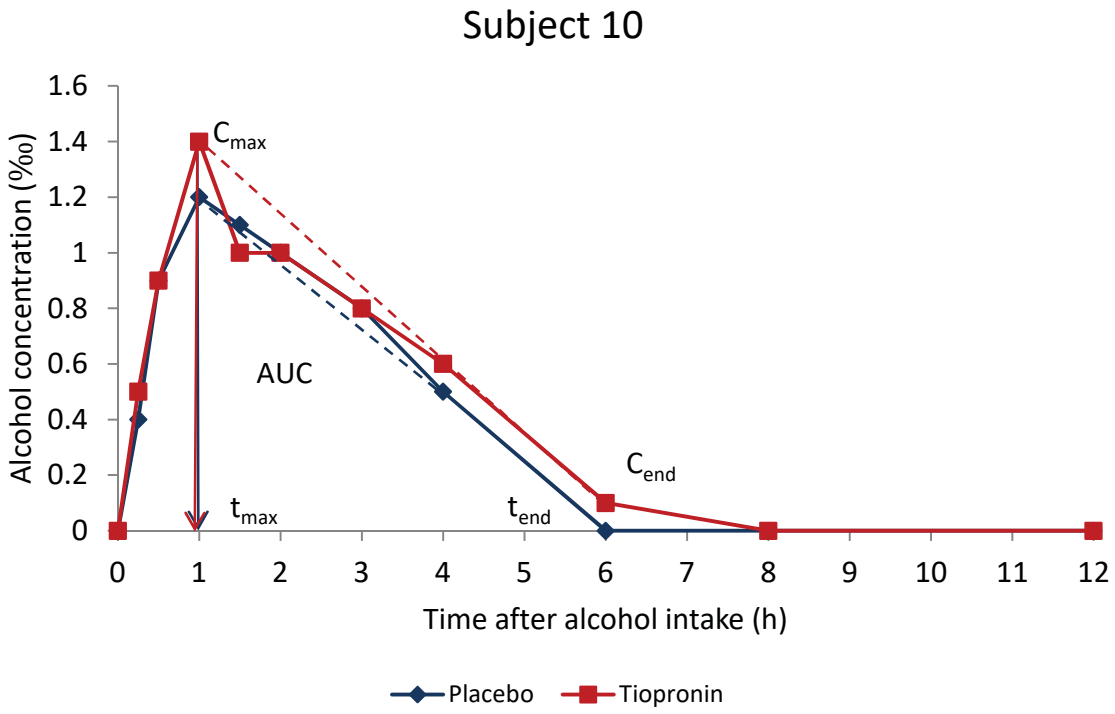


Fig. 16: Concentration-time profile subject 10

Subject 10	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Placebo	1	1.2	3.89	0.23
Tiopronin	1	1.4	4.31	0.26

Table 9: Kinetic parameters subject 10

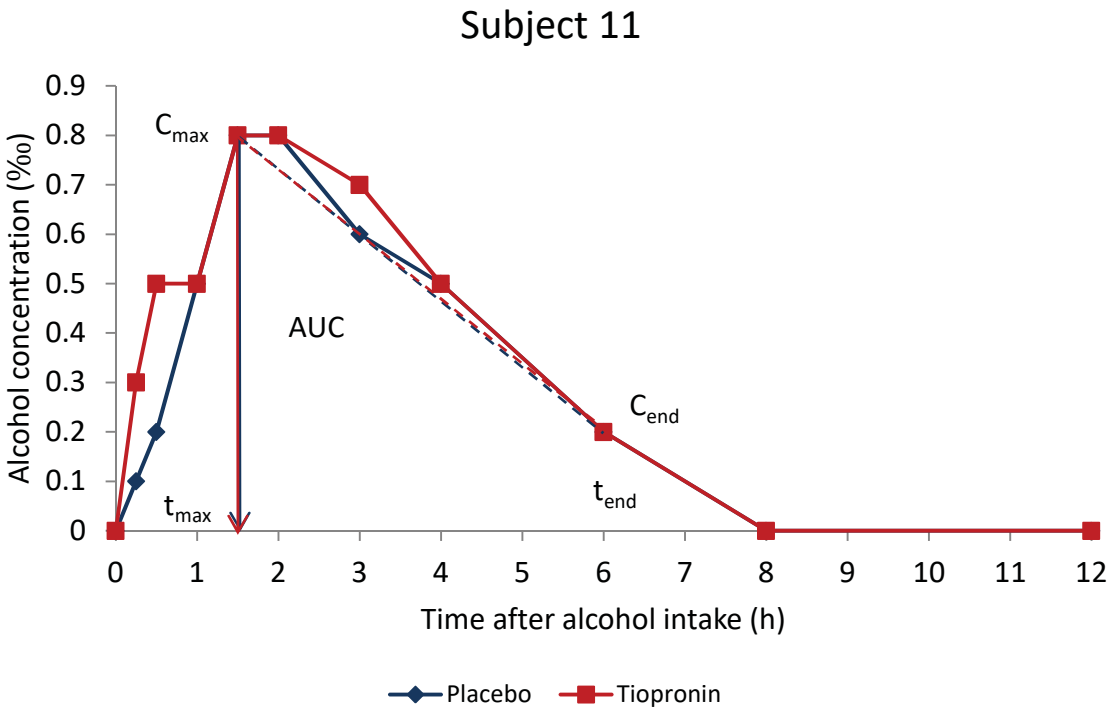


Fig. 17: Concentration-time profile subject 11

Subject 11	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Placebo	1.5	0.8	3.10	0.15
Tiopronin	1.5	0.8	4.36	0.15

Table 10: Kinetic parameters subject 11

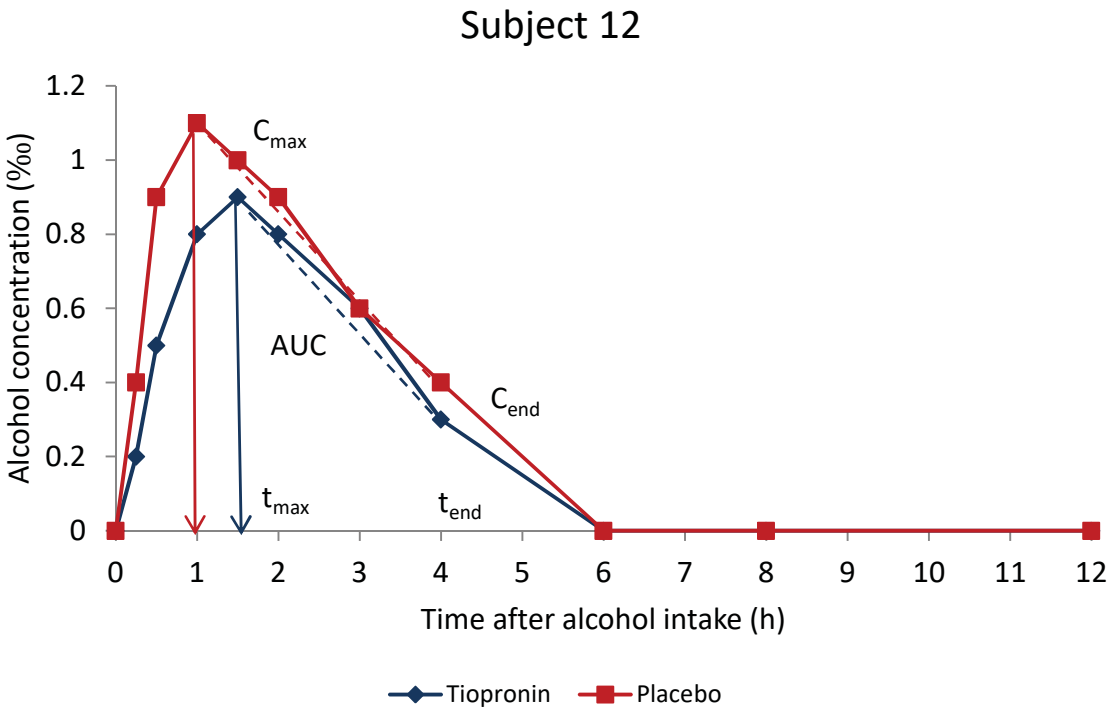


Fig. 18: Concentration-time profile subject 12

Subject 12	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Tiopronin	1.5	0.9	2.74	0.24
Placebo	1.0	1.1	3.36	0.23

Table 11: Kinetic parameters subject 12

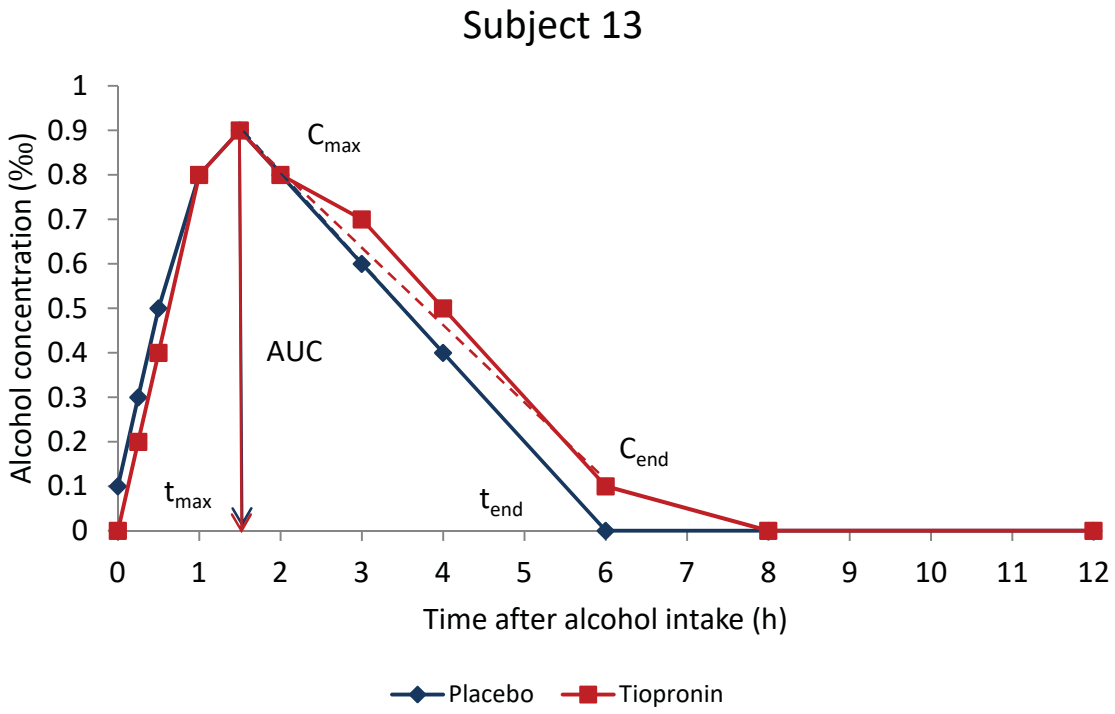


Fig. 19: Concentration-time profile subject 13

Subject 13	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Placebo	1.5	0.9	2.93	0.20
Tiopronin	1.5	0.9	3.30	0.18

Table 12: Kinetic parameters subject 13

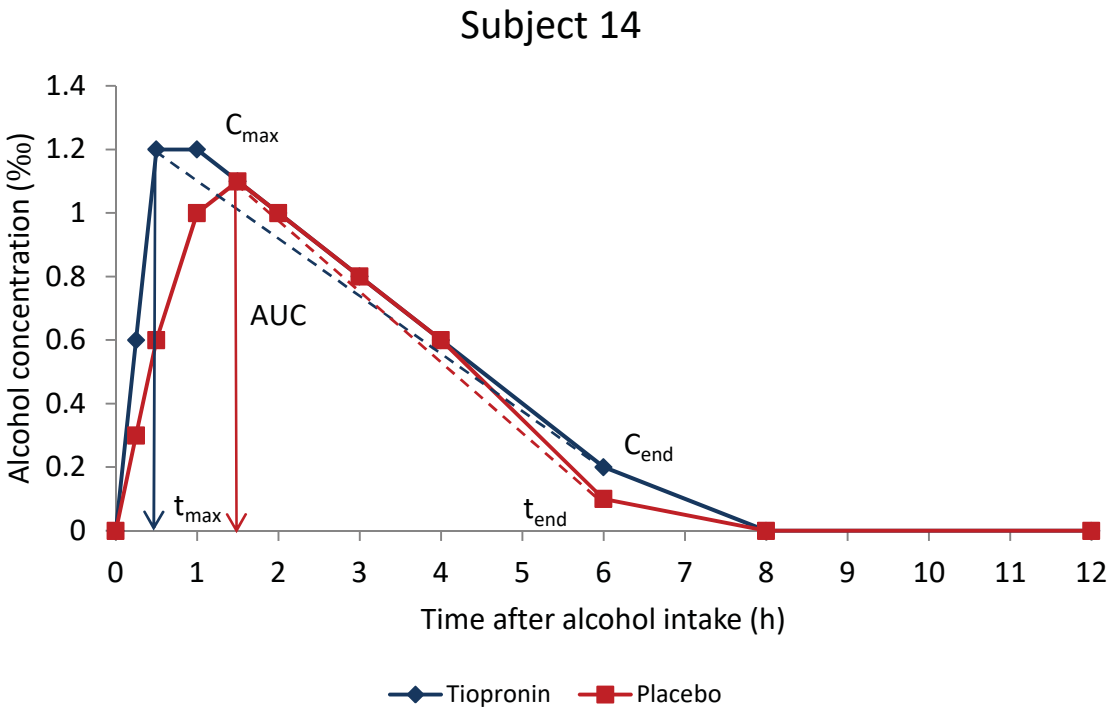


Fig. 20: Concentration-time profile subject 14

Subject 14	t_{max} (h)	C_{max} (‰)	AUC	k (‰/h)
Tiopronin	0.5	1.2	4.6	0.20
Placebo	1.5	1.1	4.0	0.22

Table 13: Kinetik parameters subject 14

10.6.1 Differences in between categories of covariates

10.6.1.1 Age

		Age years	Mean	Standard deviation	Mean difference	Standard error	95% Confidence interval		Sign. p
							lower	upper	
Period 1	t_{max}	< 25	0.9444	0.3909	-0.1806	0.25039	-0.7317	0.3705	0.486
		≥25	1.125	0.4787					
	C_{max}	< 25	1.0778	0.1716	0.0778	0.0951	-0.1316	0.2871	0.431
		≥25	1	0.1155					
	AUC	< 25	3.8181	0.6153	0.2337	0.3571	-0.5524	1.0197	0.526
		≥25	3.5844	0.5342					
	k	< 25	0.1934	0.0371	0.0084	0.0212	-0.0383	0.055	0.701
		≥25	0.185	0.03					
Period 2	t_{max}	< 25	1.0556	0.3005	-0.0694	0.1728	-0.4498	0.3109	0.695
		≥25	1.125	0.25					
	C_{max}	< 25	1.1667	0.1658	0.1167	0.1009	-0.1054	0.3387	0.272
		≥25	1.05	0.1732					
	AUC	< 25	4.3028	0.7557	0.4122	0.4457	-0.5689	1.3932	0.375
		≥25	3.8906	0.7032					
	k	< 25	0.2003	0.0394	0.0159	0.0211	-0.0305	0.0623	0.467
		≥25	0.1844	0.0194					

Table 32: Results of t-test for differences between age groups

10.6.1.2 Gender

		Sex	Mean	Standard deviation	Mean difference	Standard error	95% Confidence interval		Sign. p
							lower	upper	
Period 1	t_{max}	female	1	0.3162	0	0.2372	-0.5221	0.5221	1
		male	1	0.5					
	C_{max}	female	1.0833	0.1472	0.0547	0.0892	-0.1415	0.251	0.552
		male	1.0286	0.1704					
	AUC	female	3.7979	0.6187	0.0961	0.3358	-0.6429	0.8351	0.78
		male	3.7018	0.5905					
	k	female	0.2044	0.0386	0.0253	0.0182	-0.0148	0.0655	0.192
		male	0.1791	0.0267					
Period 2	t_{max}	female	1.0833	0.2041	0.0119	0.1611	-0.3427	0.3665	0.942
		male	1.0714	0.345					
	C_{max}	female	1.2167	0.1169	0.1596	0.0864	-0.0307	0.3498	0.092
		male	1.0571	0.1813					
	AUC	female	4.4312	0.8343	0.4741	0.4038	-0.4147	1.363	0.265
		male	3.9571	0.6212					
	k	female	0.2152	0.0395	0.0367	0.0167	0	0.0734	0.05
		male	0.1785	0.0187					

Table 33: Results of t-test for differences between differences betweenfemales and males

10.6.1.3 BMI

		BMI kg/m^2	Mean	Standard deviation	Mean difference	Standard error	95% Confidence interval		Sign. p
							lower	upper	
Period 1	t_{max}	< 25	1.0625	0.4173	0.1625	0.2381	-0.3616	0.6865	0.509
		≥25	0.9	0.4183					
	C_{max}	< 25	0.975	0.1389	-0.205	0.0694	-0.3577	-0.0523	0.013
		≥25	1.18	0.0837					
	AUC	< 25	3.4953	0.5056	-0.6522	0.2839	-1.277	-0.0274	0.042
		≥25	4.1475	0.4842					
	k	< 25	0.1809	0.0395	-0.0258	0.0187	-0.0669	0.0154	0.195
		≥25	0.2067	0.0149					
Period 2	t_{max}	< 25	1.0625	0.3204	-0.0375	0.1648	-0.4001	0.3251	0.824
		≥25	1.1	0.2236					
	C_{max}	< 25	1.0875	0.1727	-0.1125	0.0955	-0.3227	0.0977	0.264
		≥25	1.2	0.1581					
	AUC	< 25	4.1141	0.8757	-0.1609	0.4363	-1.1212	0.7993	0.719
		≥25	4.275	0.5198					
	k	< 25	0.1848	0.0342	-0.0276	0.0187	-0.0689	0.0136	0.168
		≥25	0.2124	0.0305					

Table 34: Resluts of t-test for differences between BMI groups

10.6.2 Individual results in ZVT

Sequence	Subject number	Initials	Period 1			Period 2		
			ZVT 0h	ZVT 2h	Diff. ZVT 2-0 h	ZVT 0h	ZVT 2h	Diff. ZVT 2-0 h
A-B	1	ME	2.58	2.89	0.31	3.22	2.91	-0.31
	2	CP	2.3	2.66	0.36	2.91	2.92	0.01
	5	LB	2.75	2.78	0.03	2.95	2.84	-0.11
	8	MN	2.99	3.03	0.04	3.74	3.55	-0.19
	12	JS	2.82	2.78	-0.04	3.21	3.38	0.17
	14	JV	2.37	2.56	0.19	2.95	3.04	0.09
	Mean		2.635	2.783	0.148	3.163	3.107	-0.057
B-A	3	PC	2.42	2.77	0.35	2.97	3.16	0.19
	4	CB	2.18	2.61	0.43	2.73	2.98	0.25
	6	PM	3.28	3.45	0.17	3.66	3.58	-0.08
	7	FS	2.15	2.49	0.34	2.95	2.92	-0.03
	10	AN	2.3	2.41	0.1	3.05	2.87	-0.18
	11	JW	2.67	2.91	0.24	3.21	3.16	-0.05
	13	GS	2.69	2.89	0.2	3.44	3.25	-0.19
	Mean		2.527	2.79	0.262	3.144	3.131	-0.013
Total mean			2.577	2.787	0.21	3.153	3.12	-0.033

Table 35: Individual results in ZVT

10.6.3 Individual results of alcohol breath measurements

Sequence	Subject number	Initials	Period 1			Period 2		
			Acet-aldehyde 0h	Acet-aldehyde 2h	Acet-aldehyde 4h	Acet-aldehyde 0h	Acet-aldehyde 2h	Acet-aldehyde 4h
A-B	1	ME	0	0.08	0.151	0.019	0.044	0.094
	2	CP	0.016	0.584	0.841	0	0.24	0.251
	5	LB	0.047	2.253	1.007	0	1.248	1.174
	8	MN	0.013	0.47	0.571	0.032	0.585	0.805
	12	JS	0	1.073	1.345	0.04	1.098	1.519
	14	JV	0	1.078	0.926	0	0.592	0.406
	Mean		0.0127	0.9232	0.8068	0.0152	0.6345	0.7082
B-A	3	PC	0.021	0.525	0.411	0	0.719	0.763
	4	CB	0.002	0.45	0.324	0	0.406	0.312
	6	PM	0.058	1.138	1.216	0.013	0.766	1.09
	7	FS	0	0.548	0.464	0.096	0.674	1.545
	10	AN	0	0.516	0.571	0.032	1.114	0.929
	11	JW	0	0.961	0.831	0.081	0.545	0.634
	13	GS	0	0.259	0.257	0	0.442	0.395
	Mean		0.0116	0.6281	0.582	0.0317	0.6666	0.8097
Total mean			0.0121	0.7643	0.6858	0.0241	0.6518	0.7629

Table 36: Individual results of alcohol breath measurements

10.7 ADVERSE EVENTS

Subject no.	1	2	4	5	6	10	14
Date of Birth	10.03. 1993	15.08. 1994	20.03. 1987	18.06. 1992	10.11. 1991	09.01. 1992	13.11. 1996
Heigh (m)	189	164	193	158	176	174	172
Weight (kg)	101.5	56	103	57	67	83	84
Sex	m	f	m	f	f	f	f
Adverse event during a study day	unspecific lumbar complaints	vertigo	no	no	fainting during blood drow	no	headache, nausea, vomitting
Adverse event after the study days	no	no	atypical lymphocytes, after laboratory assesments suspicion of a non-symptomatic mononucleosis	slight increased leukocytes and ESR (=BSG)	no	cystitis	no
Serious	no	no	no	no	no	no	no
Intake of tiopronin	14.11. 2015	14.11. 2015	21.11. 2015	14.11. 2015	21.11. 2015	21.11. 2015	14.11. 2015
Time between intake of tiopronin and AE	3 h	7 days	4 days	26 days	-7 days	12 days	8 h
Start	14.11. 2015	21.11. 2015	25.11. 2015	10.12. 2015	14.11. 2015	03.12. 2015	14.11. 2015
End	14.11. 2015	21.11. 2015	21.01. 2016	03.02. 2016	14.11. 2015	05.12. 2015	14.11. 2015
Intensity	mild	mild	mild	mild	moderate	mild	moderate
Relationship to study drug	unknown	unknown	not related	not related	not related	not related	unknown
Actions taken	none	other treatment	none	additional medication	other treatment	additional medication	additional medicatio n
Outcome	reversible	reversible	reversible	reversible	reversible	reversible	reversible

Table 37: Individual adverse event listing

Adverse Events

	Adverse Event (per line only one single event!)	Serious	Start End	Intensity	Relationship to study drug	Actions taken	Outcome
1	unspez. Reaktion bei bds. 08.39 - 10.00	☐ yes ☒ no	14/11/15 dd / mm / yy 14/11/15 dd / mm / yy	☒ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☒ unknown ☐ not related	☒ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☒ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
2		☐ yes ☐ no	___/___/___ dd / mm / yy ___/___/___ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
3		☐ yes ☐ no	___/___/___ dd / mm / yy ___/___/___ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
4		☐ yes ☐ no	___/___/___ dd / mm / yy ___/___/___ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown

MIT GESUNDHEIT GMBH*

Study-No.: MIT-TIO-01 Page: 35/39

Subject No.: 02 CRF-No.: ___

Date: 21/11/15 Subject Initials: CP

Adverse Events

	Adverse Event (per line only one single event!)	Serious	Start End	Intensity	Relationship to study drug	Actions taken	Outcome
1	unwohlsein, Schwindel	☐ yes ☒ no	21/11/15 dd / mm / yy 21/11/15 dd / mm / yy 15:00	☒ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☒ unknown ☐ not related	☐ none ☐ withdrawal ☐ additional medication ☒ other treatment ☐ subject excluded	☒ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
2		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
3		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
4		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown

MIT GESUNDHEIT GMBH*

Study-No.: MIT-TIO-01 Page: 1/1

Subject No.: 04 CRF-No.: ___

Date: 25/11/15 Subject Initials: CB

Adverse Events

	Adverse Event (per line only one single event!)	Serious	Start End	Intensity	Relationship to study drug	Actions taken	Outcome
1	atypical lymphocyte	☐ yes ☒ no	25.11.15 dd / mm / yy 27.11.15 dd / mm / yy	☒ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ unknown ☒ not related	☒ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☒ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
2		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
3		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
4		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown

10/12/15 gesund.

Adverse Events

	Adverse Event (per line only one single event!)	Serious	Start End	Intensity	Relationship to study drug	Actions taken	Outcome
1	Infektion	☐ yes ☐ no	25/11/15 dd / mm / yy 2_/__/__ dd / mm / yy 10/12/15 gesund	☒ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ unknown ☒ not related	☒ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☒ unknown
2		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
3		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
4		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown

Adverse Events

	Adverse Event (per line only one single event!)	Serious	Start End	Intensity	Relationship to study drug	Actions taken	Outcome
1	faintig during blood draw at 12^{29} – 12^{30} $72^{93/50}$	☐ yes ☒ no	14.11.15 dd / mm / yy 14.11.15 dd / mm / yy	☐ mild ☒ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ unknown ☒ not related	☐ none ☐ withdrawal ☐ additional medication ☒ other treatment ☐ subject excluded	☒ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
2		☐ yes ☐ no	___/___/___ dd / mm / yy ___/___/___ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
3		☐ yes ☐ no	___/___/___ dd / mm / yy ___/___/___ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
4		☐ yes ☐ no	___/___/___ dd / mm / yy ___/___/___ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown

Adverse Events

(A) Treatment by house physician

	Adverse Event (per line only one single event!)	Serious	Start End	Intensity	Relationship to study drug	Actions taken	Outcome
1	Cystitis	☐ yes ☒ no	03.12.15 dd / mm / yy 05.12.15 dd / mm / yy	☒ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ unknown ☒ not related	☐ none ☐ withdrawal ☒ additional medication (A) ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☒ unknown
2		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
3		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
4		☐ yes ☐ no	__/__/__ dd / mm / yy __/__/__ dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown

Adverse Events

	Adverse Event (per line only one single event!)	Serious	Start End	Intensity	Relationship to study drug	Actions taken	Outcome
1	Headache Vomiting	☐ yes ☒ no	14/11/15 dd / mm / yy 14/11/15 dd / mm / yy	☐ mild ☒ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☒ unknown ☐ not related	☐ none ☐ withdrawal ☒ additional medication ☐ other treatment ☐ subject excluded 30 drops Mirtazapin	☒ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
2		☐ yes ☐ no	dd / mm / yy dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
3		☐ yes ☐ no	dd / mm / yy dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown
4		☐ yes ☐ no	dd / mm / yy dd / mm / yy	☐ mild ☐ moderate ☐ severe	☐ definite ☐ probable ☐ possible ☐ no relationship ☐ unknown	☐ none ☐ withdrawal ☐ additional medication ☐ other treatment ☐ subject excluded	☐ reversible ☐ improved ☐ unchanged ☐ worsened ☐ died ☐ unknown

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