Short title: EARLY PRO-TECT Alport

Study medication: Ramipril (Delix®) tablets containing 2.5 mg Ramipril, Manufacturer: Sanofi-Aventis

Eudra-CT Number: 2010-024300-10

Register-Number: 00814 (UMG-Reg.-Nr.)

ClinicalTrials.gov (NCT01485978)

Clinical study report (gemäß ICH E3 – *ANNEX I*)

Version 1.0, March 19, 2020

Sponsor of the clinical trial:

Georg-August-Universität Göttingen, Stiftung Öffentlichen Rechts,

Universitätsmedizin Göttingen

Coordinating investigator:

Prof. Dr. Oliver Gross

Author of the clinical study report:

Prof. Dr. Oliver Gross
Klinik für Nephrologie und Rheumatologie, Universitätsmedizin Göttingen.
Robert-Koch-Str. 40, 37075 Göttingen

Study start – End of study

First patient in: 07.05.2012 – End of study: 26.03.2019

1) Name of sponsor / company	Name: Rainer Bredenkamp Institut: Georg-August-Universität Göttingen, Stiftung Öffentlichen Rechts, Universitätsmedizin Göttingen. Adresse: Robert-Koch-Straße 40, D-37075 Göttingen Tel.: +49 551/39-171347 Fax: +49 551/39-171344 E-Mail: sz-umg.sponsor-qm@med.uni-goettingen.de
2) Name of finished product	Ramipril (Delix®) tablets
3) Name of active substance	Ramipril 2.5 mg tablet
4) Individual Study Table	N/A
5) Title of study	Early Prospective Therapy Trial to Delay Renal Failure in Children with Alport Syndrome. An overview of the rationale for study protocol amendments is described in appendix 1.
6) Coordinating investigator	Name: Prof. Dr. Oliver Gross. Einrichtung: Klinik für Nephrologie und Rheumatologie, Universitätsmedizin Göttingen. Adresse: Robert-Koch-Str. 40, 37075 Göttingen. Tel.: +49 (0) 551 39 8912 Fax: +49 (0) 551 39 8906 Email: gross.oliver@med.uni-goettingen.de
7) Study centres	see appendix 3
8) Publication	*Kidney Int*, in press 2020 DOI: 10.1016/j.kint.2019.12.015
9) Study period	First patient in: 07.05.2012 Last patient in: 30.09.2015
10) Phase of development	Phase 3
11) Objectives and endpoints	This study aimed to determine the safety and efficacy of the ACEi Ramipril in delaying disease progression in patients with early stages of Alport Syndrome, in a setting of multi-centre, randomised, placebo-controlled, patient- and investigator-blind trial. <u>Study Endpoints:</u> Primary Efficacy Endpoint: Time to progression of Alport Syndrome to the next disease level under Ramipril treatment compared to placebo, for all randomised patients. Primary Safety Endpoint: Incidence of adverse drug events (ADEs, e.g., angioedema, acute renal failure, hyperkalaemia) under Ramipril treatment before disease progression compared to placebo before disease progression, for all randomised patients. Secondary Efficacy Endpoint: Albuminuria after end of treatment corrected for baseline albuminuria for patients randomised to receive Ramipril compared to placebo. Secondary Safety Endpoint: Incidence of ADEs (e.g., angioedema, acute renal failure, and hyperkalaemia) during treatment for patients randomised to receive Ramipril compared to placebo.

12) Methodology	The type IV collagen disease Alport syndrome (AS) is the second most common monogenic cause of end-stage renal failure (ESRF), and is responsible for almost 4% of chronic kidney disease (CKD) in adults. AS is caused by variants in the COL4A3, COL4A4 and COL4A5 genes, which encode for the $\alpha 3$, $\alpha 4$, and $\alpha 5$ chains of type IV collagen. The defective type IV collagen leads to basement membrane defects in the inner ear, eye, and glomerular basement membrane (GBM), leading to ESRF early in life (median 22 years in Europe). In a mouse model of AS, time to ESRF can be doubled if therapy with the angiotensin-converting enzyme inhibitor (ACEi) ramipril is started before the onset of proteinuria. This effect in mice confers only a small benefit if therapy after progressive proteinuria has taken hold. Registry data demonstrated that ACEi also delay ESRF in humans with AS in a time-dependent manner. Treatment starting in CKD stage 3 or 4 delays ESRF by a median of 3 years, while treatment starting in CKD 2 delays ESRF by a median of 18 years, leaving open the question of whether an even earlier start (CKD 0 or 1) is even more effective while remaining safe. Several features of AS pathogenesis facilitate efforts to address this question. First, the evolutionarily highly conserved type IV collagen in mammals allowed preclinical therapeutic approaches in mice with AS. The developmental switch of collagens in childen with AS allows a “window of opportunity” to initiate treatment with a “the earlier-the-better” potential before structural harm to the GBM has been established. Second, AS can be diagnosed accurately by genetic testing and has a clearly defined course starting with hematuria, microalbuminuria, and proteinuria progressing to renal fibrosis.6 Awareness of family history of renal failure improves adherence to the study protocol. The unmet medical need allowed this trial to have a long treatment period of randomized versus placebo, which is unique in a pediatric trial in a serious disease. Finally, the pros and cons of renin-angiotensin-aldosterone (RAAS) blockade have been extensively studied in adults. However, in children with CKD, the ESCAPE trial published in 2009, is still the only large clinical trial evaluating the effect of RAAS blockade in conventional versus intensified blood pressure control. Recently, post hoc analyses of the ESCAPE trial showed that early proteinuria reduction by ramipril predicted (improved) renal survival in children with CKD. The baseline characteristics in the ESCAPE trial with very low estimated glomerular filtration rate (eGFR) and the rationale (therapy to delay further kidney damage) are very different to the EARLY PRO-TECT Alport trial. Here, we tested the hypothesis that preemptive therapy in children with AS prior to ultrastructural kidney damage is safe and more efficient than later onset of therapy. This question could not be sufficiently answered in registries to justify treatment recommendations in toddlers. As a consequence, this trial is the first randomized and placebo-controlled study to investigate safety and nephroprotective properties of RAAS-blockade in children. Prospectively, an evidence synthesis with observational data was planned including patients whose parents refused randomization and who were treated open label, and untreated patients prospectively followed in the US Alport-registry. This preemptive approach could benefit most patients in early stages of glomerular kidney diseases. Alport Syndrome levels that describe the extent of renal damage and loss of function are defined as: • 0 Microhematuria without microalbuminuria (usually at birth),

	• I Microalbuminuria (30-300 mg albumin/gCrea), • II Proteinuria >300 mg albumin/gCrea, • III >25% decline of normal renal function (creatinine clearance), • IV End stage renal failure (ESRF). After diagnosis, eligible patients with Alport Syndrome stages 0 and I were randomly assigned at a 2:1 ratio to receive once daily oral Ramipril or placebo for at least 3 years (or up to 6 years in the extended treatment period). Randomised patients who progressed to the next disease level during the treatment period were unblinded, and Ramipril treatment was initiated, if applicable (in patients, who were previously treated with placebo). The aim was to treat the recruited Alport patients levels 0, I and II open label with Ramipril, including previously treated as well as un-treated patients who, or whose parents/legal guardian refuse randomisation after eligibility was confirmed. All patients, including the non-randomized children, underwent study-specific procedures according to the study schedule regardless of the treatment/ randomisation. •
13) Number of patients	Patients (planned): see Figure 1 Patients actually randomized: see Figure 1 Patients treated and analyzed as per protocol: see Figure 1
14) Diagnosis and main criteria for inclusion and exclusion	Inclusion Criteria: • Age: ≥24 months and <18 years at screening. • Gender: Male and female patients to be included. • Definitive diagnosis of Alport syndrome: Kidney biopsy (patient or affected relative/s), and/or mutation analysis (hemizygous X chromosomal or homozygous autosomal-recessive) and assessment of criteria for clinical diagnosis (haematuria, positive family history regarding kidney diseases, ocular changes, labyrinthine hearing loss). • Alport syndrome levels 0, I or II at screening (microhaematuria without microalbuminuria or microalbuminuria [30-300 mg albumin/gCrea] or proteinuria >300 mg albumin/gCrea with GFR>80ml/min). Patients with Alport level II did not randomized but were treated as an open label. • Assent from patient and informed consent from parents/legal guardian. Exclusion Criteria: • Uncertain diagnosis or variants of Alport syndrome such as a heterozygous carrier. • Alport syndrome levels III, or IV (creatinine clearance <80 mL/min, or end stage renal failure [ESRF]). • Known allergies or intolerances to ramipril or related compounds. • Known contraindication for ACEi-therapy • Additional chronic renal, pulmonary or cardiac diseases • Pregnancy and lactation

15) Test product	Ramipril (Delix®) 2.5 mg tablets The oral, once daily dose of ramipril was uptitrated from 1 mg/m2 to the target maximum dose of 6 mg/m2 body surface area in two-monthly intervals. During the trial, the uptitration of ramipril was well tolerated. Dosages were not different between groups (mean 4.4±1.1 mg/m2 placebo, 4.5±0.9 mg/m2 ramipril, 4.8±1.0 mg/m2 in the open arm). Dosages in our normotensive children were very similar to the high dosages reached in the ESCAPE trial with hypertensive children. For the used chargen numbers see appendix 4.
16) Duration of study	Treatment period 36 months (minimum) to 72 months (maximum) plus 6 months follow up
17) Reference therapy, dose and mode of administration	Placebo tablets, same dose as Ramipril and same mode of administration. For the used chargen numbers see appendix 4.
18) Criteria for evaluation: Efficacy, Safety	<u>Efficacy:</u> Efficacy was assessed by laboratory tests (blood, urine), height, and weight. After completion of the treatment period, patients were advised to undergo a hearing and an eye examination. Additional serum and urine samples were taken for the development of serum and urinary markers of renal disease progression. A blood sample for DNA extraction were taken if indicated to confirm the patient's Alport mutation (genetic testing was not part of this study). After completion of the treatment period, treatment of Alport syndrome was continued and initiated, respectively, at the investigator's discretion and the patients and/or parents' wish. A follow-up examination was done after 6 months from the last study visit or after premature termination of study participation. <u>Safety:</u> Safety was assessed by recording adverse events, laboratory parameters, vital signs, physical examination, and concomitant medication. Pharmacological-toxicological evaluation was not applicable. Risks included potential side effects of ACEis. Significant side effects were expected to include dry cough, hyperkalemia, hypotension, angioedema, acute renal failure, cataract, and hepatitis. Major adverse drug reactions were expected in less than 1% of the study participants. Ramipril is contraindicated during pregnancy and breast-feeding. The antihypertensive and antiproteinuric effect of ramipril has been tested in other renal diseases. Concomitant medication was planned to be avoided during the study unless required to treat an AE or for the treatment of an on-going medical problem. Medications affecting blood pressure and immune-suppressants were avoided in the randomized arm of this study. Non-steroidal anti-inflammatory drugs (NSAID) were allowed to be administered for no more than 1 week e.g., for the treatment of an acute injury. For randomised patients treated in a double blind fashion, emergency codes were available to the investigator. A code, which revealed the treatment group for a specific study patient, was opened during the study only if the choice of treatment depends on the study subject's therapy assignment or if the patient progresses to the next disease

	level.
19) Statistical methods	Statistical analysis of this study were the responsibility of the Dept. Medical Statistics, Universitätsmedizin Göttingen, Georg-August Universität. The baseline value for glomerular filtration rate (GFR) and albuminuria were the mean value of the Screening visit and the Baseline visit. Values at the end of the treatment period were computed as repeat measurement of serum creatinine, creatinine clearance, and albuminuria on two different days within one week. Safety analyses was performed for the full analysis set, i.e. the intent-to-treat (ITT) population. Efficacy analysis was performed for the ITT population, and supporting analyses will be conducted on the per protocol population (PPP). Additional exploratory analyses of the data was conducted as deemed appropriate. A continuous risk-benefit assessment was performed by the independent Data and Safety Monitoring Board (DSMB). The analyses was also stratified by age groups. In case of significant safety risks, study participation might be terminated for the individual patient or for the entire study. The primary efficacy endpoint 'time to progression of Alport Syndrome to the next disease level under Ramipril treatment in compared to placebo, for randomised patients' was assessed in 6-monthly intervals over the treatment period. A proportional hazards model for interval censored data was applied with effects for treatment, trial site, and proteinuria at start of therapy. If indicated, additional potentially confounding variables such as genotype and age were added to the model. An estimate of the treatment effect was reported in terms of the hazard ratio with 95% confidence interval and p-value testing the null hypothesis of no effect. The secondary efficacy endpoint 'albuminuria after end of treatment corrected for baseline albuminuria for patients randomised to receive ramipril compared to placebo' will be analysed by means of an analysis of covariance (ANCOVA) with treatment, trial site, and proteinuria at start of therapy as factors and baseline albuminuria as covariate. The treatment effect estimate in terms of a mean difference will be reported with 95% confidence interval and p-value testing the null hypothesis of no treatment effect. The secondary safety endpoint 'incidence of ADEs (e.g., angioedema, acute renal failure, hyperkalaemia) during treatment for patients randomised to receive Ramipril compared to placebo' will be analysed in the same way as the primary safety analyses. Results will also be stratified by age groups.
20) Summary - Conclusions	In conclusion, our study reaches the most important co-primary endpoint, safety: preemptive ramipril therapy prior to kidney damage in as-yet-oligosymptomatic children with AS aged two years and older is safe. In conclusion, future treatment recommendations will carefully consider whether nephroprotective therapy should be started in children with AS as early as in the stage of micro-hematuria, even prior onset of micro-albuminuria in most cases: our trial provides very important evidence for this decision, however, initiation of therapy remains an individual risk-benefit challenge for parents and the caregiver while taking the family history of early renal failure into account. Our study aims to achieve a conceptual shift toward preemptive organoprotective therapy with ACEis in the early CKD stages. This might apply for most glomerular CKDs, as identification of heterozygous variants in Alport genes with a range of glomerular

	pathologies, including FSGS and diabetic kidney disease increases. Thus, our study fills a very important gap in the assessment of glomerular hematuria and microalbuminuria in daily clinical practice and supports a possible approval extension for ramipril for the indications of AS and proteinuric glomerular kidney diseases in the field of pediatrics.
21) Date of report	March 19, 2020

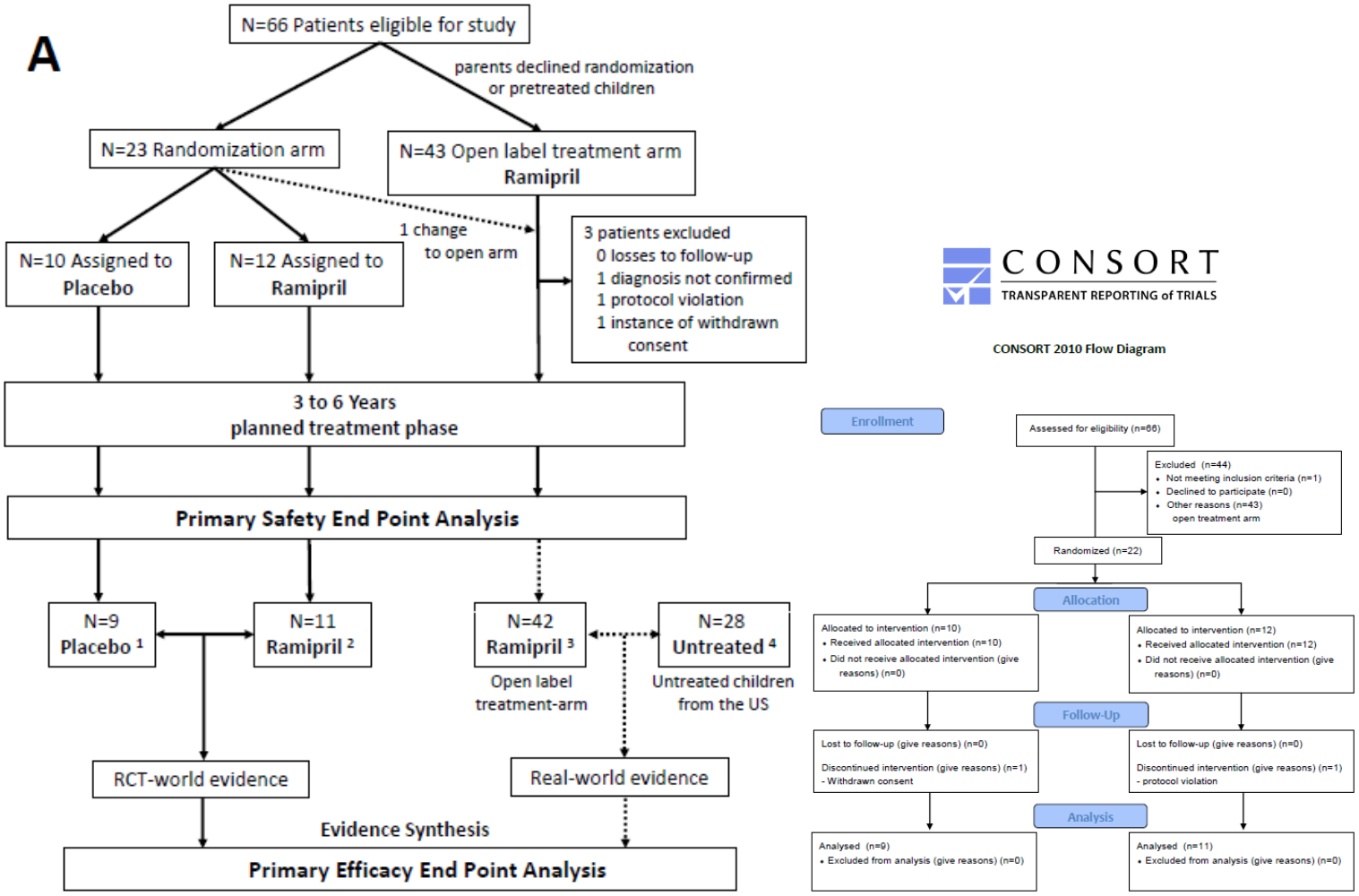


Fig. 1. Eligibility, Enrollment, Randomization

Flow diagram including the open-arm (CONSORT Flow Diagram of randomized patients provided on the right). A total of 20 randomized children completed the trial and were analyzed (randomized controlled trial evidence). In addition, 42 children in the open-ramipril treatment arm completed the trial and were compared to data from 28 untreated children from the US registry (real-world evidence) in an evidence synthesis approach.

[1] n=1 premature study discontinuation;

[2] n=1 protocol violation at baseline;

[3] n=4 premature study discontinuation, however, patient data until study discontinuation included in analysis (plus data from one patient until protocol violation);

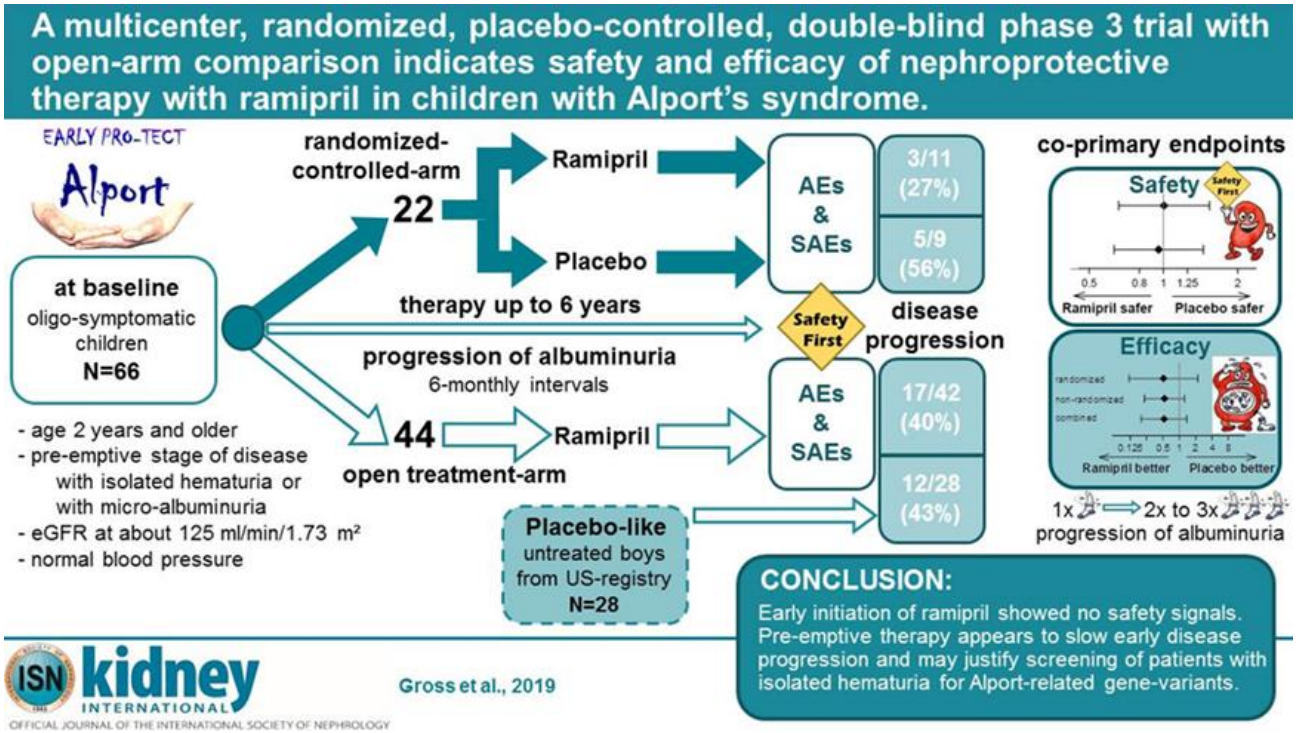
[4] untreated children from US-Alport registry (ASTOR), NCT00481130;

RCT=randomized controlled trial.

Appendix 1 Overview of the rationale for study protocol amendments

In the first year, challenges to recruitment became obvious: (1) children whose urine dipstick had been negative for albuminuria, had albuminuria in the range of 300 mg/gCrea at the more thorough laboratory examination, excluding them from randomization; (2) randomization versus placebo was rejected by parents because of their child's severe disease; (3) observational evidence for ACEi-therapy published in 2012 reduced the willingness of investigators to participate in such a long, tight-financed academic study; and (4) our efforts for expansion of the trial to outside Germany (Paris and London) failed due to very low numbers of oligosymptomatic patients eligible for randomisation in these countries (at that time, Germany intended to genetically diagnose children with AS earlier than other European countries). To make randomization more attractive, we changed to a 2:1 randomization regime in 2014. All changes in the trial-protocol were approved by the relevant parties including all ethics committees and the BfArM. To compensate for the lower number of randomized children, we switched to a flexible follow-up design expanding the treatment phase to up to 6 years. Prospective “real-world” data from untreated children from the US registry (NCT00622544) were used for evidence synthesis.

Appendix 2 Graphical summary of our trial



Appendix 3 Overview of sites

Site No	Site
01	Universitätsmedizin Göttingen, Nephrologie u. Rheumatologie, Studienbüro: 2C4 802. Klinik für Kinder- und Jugendmedizin, AWT 460, Raum 1.B4 161. Robert-Koch Str. 40 37075 Göttingen
02	Universitätsklinikum Essen Klinik für Kinderheilkunde II Hufelandstr. 55 45122 Essen
03	Clementine Kinderhospital Theobald-Christ-Str. 16 60316 Frankfurt
05	Klinik für Pädiatrische Nieren-, Leber- und Stoffwechselerkrankungen MHH Carl-Neuberg-Straße 1 30625 Hannover
06	Zentrum für Kinder- und Jugendmedizin Universitätsklinikum Heidelberg Im Neuenheimer Feld 430 Postfach 156 69120 Heidelberg
07	Klinik und Poliklinik für Kinder- und Jugendmedizin Uniklinik Köln Kerpener Str. 62 50937 Köln
09	Klinik für Kinderheilkunde und Jugendmedizin Klinikum Memmingen Bismarckstr. 23 87700 Memmingen
10	Dr. von Haunersches Kinderspital Kinderklinik und Kinderpoliklinik der Ludwig-Maximilians-Universität Lindwurmstr. 4 80337 München
11	Universitätsklinikum Rostock Kinder- und Jugendklinik KfH Nierenzentrum f. Kinder u. Jugendliche Rembrandtstraße 16/17 18057 Rostock

Site No	Site
12	Universitäts-Kinderklinik Münster Pädiatrische Nephrologie / KfH Nierenzentrum Waldeyerstr. 22 48149 Münster
13	Universitätsklinikum Klinik für Kinder- und Jugendmedizin Haus E Am Klinikum 1 07747 Jena
14	Charité –Campus Virchow-Klinikum Klinik für Pädiatrie mit Schwerpunkt Nephrologie Augustenburger Platz 1 13353 Berlin
15	Universitätsklinik Bonn Zentrum für Kinderheilkunde Pädiatrische Nephrologie Adenauerallee 119 53113 Bonn
16	Universitätsklinikum Erlangen Kinder- und Jugendklinik Loschgestraße 15 91054 Erlangen

Appendix 4 List of used chargen numbers

hergestellte Ware

Charge	Haltbarkeit	Anzahl Kruken
H1112150001	14.12.2016	500,0
H1112190002	18.12.2019	250,0
H1201180003	22.01.2020	500,0
H1201230004	22.01.2020	500,0
H1201250005	24.01.2017	500,0
H1201270006	14.12.2018	500,0
H1202020007	01.02.2017	500,0
H1202060008	05.02.2015	500,0
H1202080009	07.02.2015	500,0
H1202100010	09.02.2015	249,0
H1202140011	13.02.2017	313,0
H1406030012	03.06.2017	87,0
H1406050013	05.06.2017	22,0
H1406050014	05.06.2017	28,0
H1408250015	31.12.2016	77,0
H1408270016	31.12.2016	64,0
H1408290017	31.12.2016	64,0
H1409020019	31.12.2016	63,0
H1409040018	31.12.2016	103,0
H1505300020	31.01.2018	113,0
H1506060021	31.01.2018	77,0
H1506060022	31.01.2018	79,0
H1506070023	31.01.2018	161,0
H1701240024	31.01.2018	106,0
H1706220025	31.01.2020	196,0
H1706290026	31.01.2020	47,0
		6099,0

vernichtete Ware

Charge	Anzahl Kruken
H1112150001	334
H1112190002	188
H1201180003	468
H1201230004	398
H1201250005	383
H1201270006	läuft
H1202020007	456
H1202060008	378
H1202080009	168
H1202100010	159
H1202140011	261
H1406030012	0
H1406050013	1
H1406050014	0
H1408250015	0
H1408270016	1
H1408290017	0
H1409020019	49
H1409040018	0
H1505300020	1
H1506060021	21
H1506060022	75
H1506070023	0
H1701240024	22
H1706220025	läuft
H1706290026	läuft
	3363,0

versendete Ware

Charge	Anzahl Kruken	
H1112150001	130	
H1112190002	38	
H1201180003	11	
H1201230004	67	
H1201250005	60	
H1201270006	59	
H1202020007	17	
H1202060008	114	
H1202080009	336	*
H1202100010	93	**
H1202140011	0	
H1406030012	87	
H1406050013	21	
H1406050014	28	
H1408250015	77	
H1408270016	63	
H1408290017	64	
H1409020019	14	
H1409040018	103	
H1505300020	112	
H1506060021	53	
H1506060022	4	
H1506070023	160	
H1701240024	81	
H1706220025	151	
H1706290026	22	
	1965	

*24 Kruken über Zweitfreigabe

**12 Kruken über Zweitfreigabe

versendete Ware pro Zentrum

Zentrumsnummer	Anzahl Kruken
1	163
2	72
3	121
4	24
5	131
6	271
7	204
8	42
9	129
10	121
11	126
12	172
13	138
14	132
15	64
16	55
	1965

retournierte Ware

Charge	Anzahl Kruken
H1112150001	21
H1112190002	8
H1201180003	3
H1201230004	17
H1201250005	21
H1201270006	12
H1202020007	12
H1202060008	18
H1202080009	47
H1202100010	27
H1202140011	0
H1406030012	24
H1406050013	15
H1406050014	13
H1408250015	11
H1408270016	14
H1408290017	24
H1409020019	1
H1409040018	35
H1505300020	31
H1506060021	9
H1506060022	4
H1506070023	21
H1701240024	0
H1706220025	0
H1706290026	0
	388