RESEARCH PROTOCOL
CATERPILLAR-study
(Version 4.0 19-07-2022)

CATERPILLAR
STUDY

PROTOCOL TITLE 'The efficacy of a lock solution containing taurolidine, citrate and heparin for the prevention of tunneled central line-associated bloodstream infections in pediatric oncology patients, a randomized controlled, mono-center trial'

Protocol ID	CATERPILLAR
Short title	Efficacy of TauroLock™-Hep100
EudraCT number	Medical Device study, not applicable.
Version	4.0
Date	19-07-2022
Project leader	M.H.W.A. Wijnen, Pediatric Surgeon Princess Máxima Center for Pediatric Oncology Heidelberglaan 25 3584 CS Utrecht the Netherlands M.H.W.A.Wijnen-5@prinsesmaximacentrum.nl
Principal investigator	M.H.W.A. Wijnen, Pediatric Surgeon Princess Máxima Center for Pediatric Oncology Heidelberglaan 25, 3584 CS Utrecht, the Netherlands M.H.W.A.Wijnen-5@prinsesmaximacentrum.nl
Study coordinator	C.H. van den Bosch, PhD-student Princess Máxima Center for Pediatric Oncology Heidelberglaan 25, 3584 CS Utrecht, the Netherlands C.H.vandenbosch-4@prinsesmaximacentrum.nl +31625395632
Clinical researchers	M.D. van de Wetering, Pediatric Oncologist Princess Máxima Center for Pediatric Oncology Heidelberglaan 25, 3584 CS Utrecht, the Netherlands M.D.vandeWetering-4@prinsesmaximacentrum.nl C.P. van de Ven, Pediatric Surgeon Princess Máxima Center for Pediatric Oncology Heidelberglaan 25, 3584 CS Utrecht, the Netherlands C.P.vandeven-4@prinsesmaximacentrum.nl A.F.W. van der Steeg, Pediatric Surgeon Princess Máxima Center for Pediatric Oncology Heidelberglaan 25, 3584 CS Utrecht, the Netherlands A.F.W.vanderSteeg@prinsesmaximacentrum.nl

	Y.G.T. Loeffen, Infectious Diseases Wilhelmina Children's Hospital Heidelberglaan 100, 3584 CX Utrecht Utrecht, the Netherlands Y.G.T.Loeffen-2@umcutrecht.nl Dr. J.T. ter Bruggen and Dr. Frakking Medical Microbiologist Department of Medical Microbiology University Medical Center Utrecht Heidelberglaan 100, 3584 CX Utrecht, the Netherlands
Trial statistician	M.F. Fiocco, Professor of Statistics Princess Máxima Center for Pediatric Oncology Heidelberglaan 25, 3584 CS Utrecht, the Netherlands M.F.Fiocco@prinsesmaximacentrum.nl Leiden University Medical Center (LUMC) Albinusdreef 2, 2333 ZA Leiden, the Netherlands Mathematical Institute – Leiden University Niels Bohrweg 1, 2333 CA Leiden, the Netherlands
Sponsor	Princess Máxima Center for Pediatric Oncology Heidelberglaan 25, 3584 CS Utrecht, the Netherlands
Subsidising party	The Dutch Cancer Society (KWF) Delflandlaan 17, 1062 EA Amsterdam, the Netherlands TauroPharm GmbH August-Bebel-Straße 51, D-97297 Waldbüttelbrunn, Germany
Independent expert	Dr. Bierings Pediatric Oncologist Princess Máxima Center for Pediatric Oncology Heidelberglaan 25, 3584 CS Utrecht, the Netherlands
Trial and Data Center	Prof. Dr. M. Zwaan Head of Trial and Data Center Princess Máxima Center for Pediatric Oncology Heidelberglaan 25, 3584 CS Utrecht, the Netherlands
Pharmacy	Dr. Frederieke Engels, Pharmacist Trial Pharmacy Princess Máxima Center for Pediatric Oncology Heidelberglaan 25, 3584 CS Utrecht, the Netherlands

PROTOCOL SIGNATURE SHEET

Name	Signature	Date
Sponsor and Head of Trial and Data Center: Prof. Dr. Zwaan Head of Trial and Data Center Princess Máxima Center for Pediatric Oncology	DocuSigned by: Prof. Dr. C.M. Zwaan Naam ondertekenaar: Prof. Dr. C.M. Zwaan Reden voor ondertekening: Ik keur dit document goed Ondertekentijd: 31-aug-2022 | 9:34 PM CEST DD3367F7616B442D81ED81C48F2F2C94	31-aug-2022 | 9:34 PM CEST
Project leader: Prof. Dr. Wijnen Pediatric Surgeon Princess Máxima Center for Pediatric Oncology	DocuSigned by: Naam ondertekenaar: Prof. dr. M.H.W.A. Wijnen Reden voor ondertekening: Ik keur dit document goed Ondertekentijd: 01-sep-2022 | 8:52 AM CEST 7CB44ED1F7C843CAB8867CDE3D2DE0F2	01-sep-2022 | 8:52 AM CEST

TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)
AE	Adverse Event
AR	Adverse Reaction
ASDIN	American Society of Diagnostic and Interventional Nephrology
BSI	Bloodstream Infection
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CL	Citrate Lock
CLABSI	Central Line Associated Bloodstream Infection
CoNS	Coagulase Negative Staphylococci
CRBSI	Central Line Related Bloodstream Infection
CT	Chemotherapy
CV	Curriculum Vitae
CVAD	Central Venous Access Device
CVT	Central Venous Thrombosis
DSMB	Data Safety Monitoring Board
ERBP	European Renal Best Practice
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GMP	Good Manufacturing Practice
H-CVAD	Hickman®-Central Venous Access Device
HL	Heparin Lock
IB	Investigator's Brochure
IC	Informed Consent
ICU	Intensive Care Unit
IGJ	The Health and Youth Care Inspectorate
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
MBI-LCBI	Mucosal Barrier Injury – Laboratory Confirmed Bloodstream Infection
M-EDTA	Minocycline and Edetic Acid
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MIC	Minimal Inhibitory Concentration
MRSA	Methicillin-Resistant Staphylococcus Aureus
PL	PowerLine®
RCT	Randomized Controlled Trial
RR	Rate Ratio
(S)AE	(Serious) Adverse Event
SCT	Stem Cell Transplantation
SPC	Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)

SUSAR	Suspected Unexpected Serious Adverse Reaction
TCHL	Taurolidine Citrate Heparin Lock solution
TCL	Taurolidine Citrate Lock solution
THL	Taurolidine Heparin Lock solution
TIVAP	Totally Implantable Venous Access Port
TPN	Total Parenteral Nutrition
VMO	Voorlopige Medicatie Overdracht
WBP	Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Tunneled central venous access devices (CVAD) are fundamental in pediatric oncology for long-term venous access. (3) The incidence of central line-associated bloodstream infections (CLABSI) is high. (4) In the Princess Máxima Center, the incidence rate of CLABSI is 1.51 per 1,000 CVAD-days, CLABSIs are seen in at least 30% of the children with a CVAD, 17% of the inserted CVADs are removed early and 5% of the patients are admitted at an intensive care unit due to CLABSIs. (1) Central venous thrombosis (CVT) is another severe complication of a CVAD, with an incidence rate of 0.02-0.24 per 1,000 CVAD-days. (1, 5-8) After a review of the literature, we concluded that the taurolidine-citrate(-heparin) lock solution (TCHL) is the most promising method for the prevention of CLABSIs and CVTs. (2, 9-50) In the Netherlands, the heparin lock (HL) is the standard of care. The HL however, does not have an antimicrobial activity and its use is barely supported by literature. (9) The TCHL has anticoagulant and antimicrobial activities without reported resistance to taurolidine. (12-50) The TCHL has shown to significantly decrease the CVAD-infection incidence in hemodialysis, total parenteral nutrition, and adult oncology patients compared to citrate, heparin and saline locks (rate ratios ranged from 0.00-0.77). (12-44) In pediatric oncology patients, six studies have been performed. (45-50) Unfortunately, these studies did not deliver enough evidence to implement the TCHL in pediatric oncology patients, mainly due to the small study groups, n-total ≤ 180. (45-50) Therefore, we want to perform an open labelled randomized controlled trial (RCT) in a large patient group (n=462) so that we can finally draw conclusions on the efficacy and safety of the TCHL in pediatric oncology patients. Our goal is to increase the quality of life for children with cancer by reducing the CLABSI-rate, CVAD-removal rate, dispense of antibiotics, days of hospital/intensive care admission, and morbidity/mortality rate due to CLABSI.

Objective: To compare the efficacy of the TCHL to the HL in the prevention of tunneled CLABSIs in pediatric oncology patients.
Study design: Investigator-initiated, mono-center, open-labelled randomized controlled trial (RCT). The patients will be followed-up for 90 days in the Princess Máxima Center for Pediatric Oncology and 21 shared care centers in the Netherlands. All data will be collected in in the Princess Máxima Center for Pediatric Oncology.
Study population: Pediatric oncology patients (n=462), ranging from 0-19 years old, who will receive a tunneled CVAD in the Princess Máxima Center for Pediatric Oncology.
Intervention: Patients in the intervention study arm will receive lock solutions containing taurolidine 1.35%, citrate 4.0%, and heparin 100 IU/ml. Patients in the control study arm will receive lock solutions containing heparin 100 IU/ml. The lock solutions will be instilled with a maximum of once weekly, and a minimum of once every three weeks. In between, all CVADs will be locked with standard heparin 100 IU/ml.
Main study parameter: Incidence of CLABSI

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The expected side effects are temporarily, caused by a spill-over of citrate, and only described if the TCHL is instilled to fast or if the TCHL is accidentally flushed instead of aspirated: perioral dysesthesia, discomfort of neck and chest, dysgeusia, nausea and vomiting. (23, 34, 35, 37, 39, 40, 45, 46, 48, 50) Additionally, hypersensitivity reactions, and heparin induced thrombocytopenia are rare but possible side effects. (34) The locks will be instilled with a maximum of once weekly and a minimum of once every three weeks. For a small number of patients this means that they have to visit the Princess Máxima Center for Pediatric Oncology 1-2 times more during the follow-up period compared to patients that do not participate in the study. After every study-lock instillation, the patients will be asked to answer a questionnaire about the experience of possible side effects. Our hypothesis is that the TCHL will reduce the CLABSI rate compared to the HL. Therefore, the TCHL may reduce the administration of antibiotics, result in lower rates of CVAD removal, fewer days of hospital/ICU admission, and a reduced mortality rate due to CLABSIs compared to the HL. Additionally, patients will benefit directly from reduced and more appropriate antibiotic use, without the risk of antibiotic resistance development.(12-50)

1. AMENDMENTS

The following amendments and/or administrative changes have been made to this protocol since the implementation of the first approved version:

Amendment number	Date of amendment	Protocol version number	Type of amendment	Summary of amendment
1	06-08-2020	1.2	Non-substantial	Section 7.3 and appendix 5 and 6: Clarifications of study procedure and patientcard/stickers changed.
2	07-10-2020	1.3	Non-substantial	Section 7.3: Change patientcard/stickers.
3	03-02-2021	2.0	Substantial	Chapter 3.0: Minor formatting/spelling changes and description of expert panel. Section 4.3: Clarification of exclusion criteria. Section 5.1 and 6.6: Clarification of lock aspiration. Section 7.3, 10.2 and 12.2: Change in inclusion period. Section 7.3: Change in study procedure if patients do not visit hospital within 3 weeks. Section 3.0, 7.1.3, 7.4, 13.1, 13.7: Addition of an extra endpoint (second CVAD insertion).

				Section 7.1.3: Clarification of endpoints. Section 10.2: Clarification of informed consent procedure. Section 10.5: Removal of description of compensation fee.
4	24-08-2021	3.0	Substantial	Section 2.0, 7.1.2, 7.1.4, 9.2, appendix 5/6: Local infections added as secondary outcome. Section 2.0, 6.4, 7.1.4, 7.3, 9.2, 12.2, and appendix 5: Liver enzymes will no longer be reported.
5	19-07-2022	4.0	Substantial	Section 4.4: We clarified how to account for drop-outs at the end of the study. Section 9.1 and 9.2: Clarification of statistical analyses for primary and secondary outcomes. Appendix 7: Typo removed.

2. INTRODUCTION AND RATIONALE

Central venous access devices in pediatric oncology patients

Central venous access devices (CVAD) are fundamental in pediatric oncology. CVADs are used for stem cell transplantation (SCT), total parenteral nutrition (TPN), blood sampling, chemotherapy (CT) and other intravenous therapies. Long-term central venous access can be provided by tunneled CVADs. The most commonly inserted CVADs are the Hickman®(H)-CVADs/Powerlines® (PL) and totally implantable central venous access ports (TIVAP), these account for 94.2% of all CVADs inserted in our hospital, the Princess Máxima Center for Pediatric Oncology, Utrecht, the Netherlands. Since the official opening of the Princess Máxima Center in June 2018 approximately 35-40 CVADs per month are inserted by surgeons in the operating theatre. (1, 3)

The incidence of central line associated bloodstream infections (CLABSI) ranges between 0.1-2.3 per 1,000 CVAD-days, depending on the patient population and infection definitions used. (4) In our pediatric oncology institution a retrospective study investigating the incidence of CVAD related complications in 201 pediatric oncology patients with 307 CVADs was performed. The incidence rate of CLABSIs was 1.51 per 1,000 CVAD-days, this means that a CLABSI was observed in 29.9% of the patients who received a CVAD. (1) Another severe complication of the CVAD is a central venous thrombosis (CVT), with an incidence rate of 0.02-0.24 per 1,000 CVAD days. (1, 5-8) Both complications frequently result in high morbidity and CVAD-removal rates. Of all CVADs inserted, 17% were removed due to a CLABSI. 41.7% Of the CLABSI episodes were successfully treated with systemic antibiotic treatment (SAT), the other CLABSI episodes eventually resulted in reinfections and/or early removal of the CVAD. Five percent of the patients that received a CVAD were admitted to the intensive care unit (ICU) due to severe sepsis caused by CLABSIs. Additionally, nine cases of CVTs were observed of which four resulted in removal of the CVAD. (1)

CLABSI prevention

There are multiple strategies for the prevention of CLABSIs: e.g. education and training of healthcare providers, carefully weighing the risks and benefits of CVAD insertion, the choice of a CVAD with the minimum number of ports/lumen needed, antimicrobial/antiseptic impregnated CVADs, maximal sterile barrier precautions during insertion, skin preparation with chlorhexidine before CVAD insertion, hand hygiene, catheter site dressing regimens, use of a chlorhexidine wash for skin cleansing, frequent CVAD insertion site checks, antimicrobial CVAD lock prophylaxis, the use of needleless intravascular CVAD systems, removal of the CVAD if the CVAD is no longer required, and limiting the amount of CVAD replacements. (3, 51) In our center, a CLABSI prevention meeting is held frequently to evaluate all of the above stated strategies. Due to the conclusions from these meetings the protocols in our hospital are tightened since January 2020. The following interventions are still under discussion in these CLABSI prevention meetings (e.g. chlorhexidine-impregnated dressings, and CVAD lock prophylaxis). The efficacy and safety of chlorhexidine-impregnated dressings is a strategy that needs to be investigated in the future for patients under 18 years before implementation. However, due to the risk of localized dermatitis associated with chlorhexidine-impregnated dressings in neonate patients, we concluded that the risk would be too high to perform a study in our hospital. (3, 51, 52) Additionally, we agreed that a great deal is to be gained from CVAD lock prophylaxis. More about CVAD lock prophylaxis is described in the next paragraph.

CVAD lock prophylaxis

Lock solutions are used to prevent CVADs from CLABSIs and CVTs [Figure 1]. Different locks are available for pediatric oncology patients, e.g. locks containing vancomycin, minocycline-edetic acid (M-EDTA), ethanol, taurolidine, citrate and heparin. (2, 9)

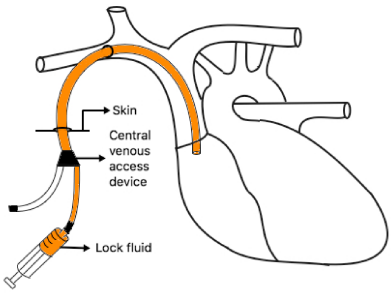


Figure 1: Lock fluid in a central venous access device

In the Netherlands, the heparin lock (HL) is the standard of care to prevent the CVAD from occlusion. (2, 9) The results of a consensus meeting in 2016 on various lock solutions showed that there is barely evidence supporting the HL. They state that the risk of CVAD occlusion is multifactorial and not solely based on blood clotting. Several studies have shown a similar effect of the HL compared to a lock solution containing regular saline. They concluded that a more important factor to prevent CVAD occlusion is an appropriate flushing technique. (9)

Vancomycin containing lock solutions are effective in the prevention of CLABSIs. Abundant antimicrobial use, however, contributes to the development of antibiotic resistance. Therefore, these locks are only recommended in high risk patients. (10, 11, 53)

M-EDTA has antimicrobial and anticoagulant activities. Until so far, one open labelled RCT and one prospective cohort study have been performed to evaluate the efficacy of M-EDTA in pediatric oncology patients. In these studies, the incidence rates of CVAD-related infections were decreased from 6.30 to 1.09, and 2.23 to 0.0 per 1,000 CVAD-days. These studies included 50 and 62 patients and compared the M-EDTA lock with the HL. These studies did not deliver enough evidence to design a study on the efficacy of M-EDTA in children. Additionally, the development of antibiotic resistance is a risk associated with the use of minocycline. (54, 55)

Another antimicrobial lock solution is ethanol. An RCT on the efficacy of the ethanol lock was performed by Wolfs et al., they included 94 pediatric oncology patients. In this study the ethanol lock did not prevent CLABSI treatment failure and it increased CVAD occlusion. (56) A second double-blinded RCT on the efficacy of a lock solution containing ethanol, in 307 pediatric oncology patients, showed a significant decrease of CLABSI from 1.46 to 0.77 per 1,000 CVAD-days without an increase of CVTs. No serious side effects were observed. However, disadvantages of the ethanol lock are the side effects (e.g. nausea, taste alteration, dizziness, blushing, and syncope), and a dwell-in time of two hours after which the lock is removed. The dwell-in time is logistically inconvenient, especially for patients. Additionally, a higher risk of occlusions is suspected with the use of ethanol, and ethanol may interfere with the polymers in some CVADs, degrading the plastic over time. (2, 9, 10)

A lock solution containing taurolidine 1.35% appears to be promising in the prevention of CLABSIs. Different lock combinations containing taurolidine are available, e.g. the

taurolidine-citrate lock (TCL), taurolidine-citrate-heparin lock (TCHL), and taurolidine-heparin lock (THL). (9-11) Taurolidine containing lock solutions offer the many advantages seen with ethanol-based solutions, while avoiding the need for an antibiotic-based solution. (10) Taurolidine containing lock solutions do not require a dwell-in time of two hours after which the lock needs to be removed and can remain in situ for maximum of 30 days (see appendix 2 for the instructions for use). The side-effects associated with taurolidine based locks (e.g. perioral dysesthesia, discomfort of neck and chest, altered taste sensations, nausea and vomiting) are rare and mainly described after the lock is accidentally flushed into the bloodstream. (23, 34, 35, 37, 39, 40, 45, 46, 48, 50) The use of the TCL and TCHL resulted in a reduction of the CVAD-infection incidence rate in haemodialysis patients, total parenteral nutrition patients, and oncology patients compared to citrate, saline or heparin locks (rate ratios (RR) ranged from 0.00-0.77). (12-50)

Evaluating the literature published on the different lock solutions our hypothesis is that lock solutions containing taurolidine are the most promising lock solutions for pediatric oncology patients.

Literature on lock solutions containing taurolidine

The majority of the literature published on the efficacy of the lock solutions containing taurolidine were based on haemodialysis patients. Two double-blinded RCTs, four open-labelled RCTs, and eight prospective cohort studies were performed in this patient group. The number of patients included ranged from 13 to 565. The incidence rates per 1,000 CVAD-days were much lower in the THL, TCL, and TCHL groups compared to the HL or CL (RRs ranged from: 0.00-0.58). See table 1 for a summary of the studies performed in haemodialysis patients. Additionally, three systematic reviews were performed concerning haemodialysis patients by Jaffer et al. (2008), Liu et al. (2014), and Kavosi et al. (2016). Jaffer et al. stated that antimicrobial lock solutions decreased CVAD-infection rates without causing significant adverse effects. Liu et al. stated that the TCL significantly reduced the risk of CVAD-related infections and specifically Gram-negative bacterial infections. Kavosi et al. stated that the TCL is superior to heparin, however due to the lack of evidence a confident decision can not yet be made. (12-26)

Table 1: Summary of studies performed in haemodialysis patients (12-26)

Author (year)	Design (adult/pediatric)	Lock type (control – intervention)	Total number of patients or CVADs* (control - intervention	CVAD infections per 1,000 CVAD-days (control – intervention), Rate Ratio (RR), p-value	Number of infections (%/patients or %/cvads*) (control – intervention)	Reduction (control – intervention)	Adverse events
Solomon et al. (2012)	Double-blinded RCT (adult)	Heparin 5,000 IU/ml - taurolidine 1.35% / citrate 4% and taurolidine 1.35% / citrate 4% / heparin 500 IU/ml	174 (34 – 34 and 106)	3.25 - 1.22 and 1.33, RR: 0.38 and 0.41 p<0.01	21 (61.8) – 7 (20.6) and 16 (15.1)	67% and 76%	Addition of heparin reduced the need for thrombolysis
Solomon et al. (2010)	Double-blinded RCT (adult)	Heparin 5,000 IU/ml - taurolidine 1.35% / citrate 4%	107 (54 – 53)	2.38 – 1.34, RR: 0.56 p=0.06 Gram-negative organisms: 1.1 – 0.2, RR: 0.18 p=0.02	23 (42.6) – 11 (20.8)	51%	Greater need for thrombolysis in taurolidine/citrate lock
Betjes et al. (2004)	Open-labelled RCT (adult)	Heparin 5,000 IU/ml – taurolidine 1.35% / citrate 4%	58 (39*-37*)	2.10 – 0.0, RR: 0.00 p=0.05	4 (10.3*) – 0 (0.0*)	100%*	No adverse events observed
Zwiech et al. (2016)	Open-labelled RCT (adult)	Heparin 5,000 IU/ml – taurolidine 1.35% / citrate 4% / heparin 500 IU/ml	53 (29 – 24)	3.44 – 0.0, RR: 0.00 p<0.05	3 (10.3) – 0 (0.0)	100%	No adverse events observed
Filiopoulos et al. (2011)	Open-labelled RCT (adult)	Heparin 5,000 – taurolidine 1.35% / citrate 4%	119 (58 – 59)	9.92 – 3.67, RR: 0.37 p=0.03	20 (34.5) – 8 (13.5)	61%	More thrombosis in taurolidine/ citrate group, not significant
Winnicki et al. (2017)	Open-labelled RCT (adult)	Citrate 4% lock – taurolidine 1.35% / citrate 4% / heparin 500 IU/ml	106 (54 – 52)	2.7 – 0.67, RR: 0.25 p<0.01	18 (33.3) – 6 (11.5)	66%	Greater need for thrombolysis in citrate lock group
Reidenberg (2018)	Prospective cohort study (adult)	Taurolidine 2.35% / citrate 3.5% / heparin 1000 IU/ml	201	0.28	13 (6.5)	n.a.	Dysgeusia (n=2)
Hulshof et al. (2017)	Prospective cohort study (pediatric)	Heparin 100 IU/ml – taurolidine 2%	23 (7 in cross-over, X-X	12.7 – 4.3, RR: 0.34 p=0.02 (cross over) 14.9 – 3.1, RR: 0.21 p<0.05	X (X) – X (X) (cross-over) 41 (X) - 8 (X)	X	No adverse events observed

			prospective cohort)	(prospective cohort)			
Murray et al. (2014)	Prospective cohort study (adult)	Heparin 5,000 IU/ml - taurolidine 1.35% / citrate 4% / heparin 500 IU/ml	565 (X tunneled CVAD patients)	Tunneled CVAD patients: 1.59 – 0.69, RR: 0.43 p<0.01	115 (X) – 43 (X)	X	No adverse events observed
Fontsere et al. (2014)	Prospective cohort study (adult)	Heparin 5,000 IU/ml - taurolidine 1.35% / citrate 4% / heparin 500 IU/ml	31 (single arm)	1.08 – 0.04, RR: 0.04 p=0.02	7 (22.6) – 1 (3.2)	86%	No adverse events observed
Allon et al. (2003)	Prospective cohort study (adult)	Heparin 5,000 IU/ml – taurolidine 1.35% / citrate 4%	50 (30 - 20)	5.6 – 0.8, RR: 0.14 p=0.02	16 (53.3) – 1 (5.0)	91%	Greater need for thrombolysis in the taurolidine/citrate group
Sodeman et al. (2001)	Prospective cohort study (adult)	Taurolidine 1.35% / citrate 4% (all patients received a Dialock access system)	70	0.29	8 (11.4)	n.a.	No adverse events observed
Taylor et al. (2008)	Prospective cohort study (adult)	Heparin 5,000 IU/ml – taurolidine 1.35% / citrate 4% / heparine 5,000 IU/ml	X (X – X)	5.2 - 0.6, RR: 0.12 p<0.01	X (X) – X (X)	89%	No adverse events observed
Geron et al. (2006) Article in Hebrew	Prospective cohort study (adult)	X - Taurolidine 1.35% / citrate 4%	13 (5 with previous infections – 8 new patients)	9.5 - 1.15 (pt with previous infections pre- and post TCL) 0.0 (new pts), RR: 0.12 and 0.00	X (X) – X (X)	X	Patency problems for which addition of heparin to lock solution in 10 patients

In total parenteral nutrition patients, two double-blinded RCTs, three open labelled RCTs, seven prospective cohort studies, and three retrospective study were performed. The number of patients included ranged from six to 270. The incidence rates per 1,000 CVAD-days were much lower in the THL, TCL, and TCHL groups compared to the HL or saline (RRs ranged from: 0.00-0.38). See table 2 for a summary of the studies performed in total parenteral nutrition patients. (27-42, 50)

Table 2: Summary of studies performed in total parenteral nutrition patients (27-42, 50)

Author (year)	Design (adult/pediatric)	Lock type (control – intervention)	Total number of patients or CVADs* (control - intervention	CVAD infections per 1,000 CVAD-days (control – intervention), Rate Ratio (RR), p-value	Number of infections (%/patients or %/cvads*) (control – intervention)	Reduction (control – intervention)	Adverse events
Wouters et al. (2018)	Double-blinded RCT (adult)	Saline 0.9% – taurolidine 2%	105 (52 – 53)	1.49 – 0.29, RR: 0.19 p<0.01	18 (34.6) – 5 (9.4)	73%	No difference in adverse events between saline and taurolidine. Dysgeusia (n=1), dizziness (n=1), erhythema exit-site (n=1) associated with the taurolidine lock.
Tribler et al. (2017)	Double-blinded RCT (adult)	Heparin 100 IU/ml – taurolidine 1.35% / citrate 4% / heparine 100IU/ml	41 (21 – 20)	1.44 – 0.33, RR: 0.23 p<0.01	7 (33.3) – 0 (0.0)	100%	Abnormal taste sensations (n=8), tingling sensation (n=3), nausea and vomiting (n=3) in taurolidine/citrate/heparin-group
Lyszkowska et al. (2019)	Open-labelled RCT (pediatric)	Standard aseptic procedures – taurolidine X / citrate X	86 (49* - 48*)	14.3 – 1.06, RR: 0.07 p=0.01	14 (28.6*) – 1 (2.1*)	93%*	No adverse events.
Klek et al. (2015)	Open-labelled RCT (adult)	Saline 0.9% – taurolidine 1.35% / citrate 4% and taurolidine 2%	30 (10 – 10 and 10)	0.0 – 0.27 and 0.0, p=1.00	0 (0.0) – 1 (10.0) and 0 (0.0)	No reduction	One occlusion in the taurolidine 2% group
Bisseling et al. (2010)	Open-labelled RCT (adult)	Heparin 150 IU/ml – taurolidine 2%	30 (14- 16)	2.02 – 0.19, RR: 0.09 p<0.01	9 (64.3) – 1 (6.3)	90%	No adverse events
Chong et al. (2020)	Prospective cross over study	Heparin X IU/ml - taurolidine 1.35% / citrate 4%	33 (TPN n=13 single arm)	11.1 – 2.9, RR: 0.26 p=0.02	X (X) – X (X)	X	Two patients experienced CVAD occlusion for which one patient switched to a TCHL. One patient experienced nausea and vomiting.
Lambe et al. (2018)	Prospective cohort study (pediatric)	Heparin - taurolidine 1.35% / citrate 4%	126 (86 – 40)	0.89 – 0.25, RR: 0.28 p<0.01	X (X) – 5 (12.5)	X	No adverse events
Jurewitsch et al (2005)	Prospective cohort study (adult)	Heparin – taurolidine 2%	7 (single arm)	10.8 – 0.8, RR: 0.07 p=missing	X (X) – X (X)	X	No adverse events
Chu et al. (2012)	Prospective cohort study (pediatric)	Heparin 10 IU/ml – taurolidine 2%	19 (single arm)	8.6 -1.1, RR: 0.13 p<0.01	47 (247.4) – 10 (52.6)	79%	No adverse events

Al-amin et al. (2013) No full-text available	Prospective cohort study (adult)	X – taurolidine 1.35% / citrate 4%	9 (single arm)	6.39 – 0.0, RR: 0.00 p=X	X (X) – X (X)	X	X
Toure et al. (2012)	Prospective cohort study (adult)	Saline 0.9% – taurolidine 1.35% / citrate 4%	15 (single arm)	6.58 – 1.09, RR: 0.17 p<0.01	36 (240.0) – 6 (40.0)	83%	No adverse events
Taniguchi et al. (2009)	Prospective cohort study (adult)	Heparin – taurolidine 1.35% / citrate 4%	6 (single arm)	0.62 – 0.16, RR: 0.25 p=0.03	21 (350.0) – 4 (66.7)	81%	Dysgeusia (n=1), perioral paraesthesia (n=1), and palpitations (n=1)
Saunders et al (2015)	Prospective cohort study (adult)	Heparin – taurolidine 1.35% / citrate 4%	22 (single arm)	5.71 – 0.99, RR: 0.17 p<0.01	42 (350.0) – 12 (54.5)	85%	No adverse events
Olthof et al. (2014)	Retrospective study (adult)	Heparin 150 IU/ml – taurolidine 2%	212 (545* - 200*)	1.10 – 0.20, RR: 0.18 p=X	464 (85.1*) – 43 (21.5*)	75%	Anaphylactic-like reaction (n=1), burning sensations (n=1), occlusion (n=1), dizziness (n=1), paraesthesia (n=1), nausea or pain (n=1), palpitations or discomfort of the chest (n=2) possibly associated with the taurolidine lock.
Wouters et al. (2018)	Retrospective (adult)	Saline - Taurolidine 2%	280 (10 – 270)	1.58 - 0.60, RR: 0.38, p=0.02	13 (130.0) - 203 (75.2)	42%	9% Of the taurolidine patients experienced mild-moderate pain, nausea, dizziness, dyspnea, palpitations, moderate pain, urticaria, pruritus, nausea and vomiting, flushes, headache, paresthesia, and edema.
Arnoriaga Rodriquez et al. (2018)	Retrospective study (adult)	X – taurolidine 2%	13 (single arm)	3.12 – 0.76, RR: 0.24 p<0.01	38 (292.3) – 4 (30.8)	90%	No adverse events

A randomized phase IV trial performed by Longo et al. in 163 adult oncology patients demonstrated a four-fold relative risk reduction of CVAD-related infections. Four CVAD-related infections were observed in 76 patients receiving a saline lock solution, one CVAD-related infection was observed in 84 patients receiving a TCL. However, this difference was not statistically significant, possibly due to power limitations. The incidence rate of CVAD-related infections in the control group was significantly lower than the one chosen as a reference in the sample size calculation. (57) Another randomized double-blinded study in 150 adult neutropenic hematological patients was performed by Gudiol et al., an incidence rate of 3.75 per 1,000 CVAD days with the TCHL compared to 8.91 per 1,000 CVAD days with the HL was found. This difference was not statisticially significant. No adverse events related to the lock solutions were observed. (44)

Six articles were published describing a decrease in the incidence rate of bloodstream infections using a TCL or TCHL in pediatric oncology patients. (45-48, 50) Simon et al. prospectively observed the incidence rate of bloodstream infections (BSI). An overall BSI incidence rate of 3.82 was found in the TCL group (n=94) compared 4.93 in the HL group (n=98), (RR: 0.77, p=0.35). However, the incidence rate of BSI due to coagulase negative staphylococci (CoNs) and methicillin-resistant *Staphylococcus aureus* (MRSA) significantly decreased from 2.30 to 0.45 per 1,000 CVAD-days, (RR: 0.20, p<0.01). Limitations of this study were: the small study group and the not-randomized study design. Additionally, CVAD-infections were defined as every bacteremia instead of CLABSI, including bacteraemia caused by infections located elsewhere in the body. (48) Ince et al. retrospectively observed a decreased incidence rate of CLABSI from 48.5% with the HL (n=33) to 22.8% with the TCL (n=79), p=0.03; CLABSI reduction of 53%. Furthermore, the duration of CVAD use per CVAD increased significantly and the incidence rate of CVAD-removal was lower in the TCL group; 81.2% vs. 33.3%. Limitations were the small study groups and retrospective study design. (47) In an open labelled RCT performed by Dumichen et al. the bacteremia incidence rate per 1,000 CVAD-days decreased from 1.30 with the HL (n=36) to 0.30 with the TCL (n=35), (RR: 0.23, p=0.03). Limitations of this study were the small study groups, that CVAD-infections were defined as every bacteremia instead of CLABSI, and that only a few CVADs were immediately locked with the lock solution after insertion of the CVAD. (45) Handrup et al. performed an open labelled RCT comparing the HL (n=65) with the TCHL (n=64). In this study, the incidence rate of CLABSI decreased significantly from 1.40 to 0.40 per 1,000

CVAD-days, (RR: 0.28, p<0.01). Especially CLABSIs caused by CoNS were reduced by 66% in the TCHL group. Other outcomes were an increased time to first CLABSI since CVAD insertion, a reduction of fungi, Gram-positive and Gram-negative microorganisms in the TCHL group, and similar rates of removal due to CVT. The incidence of overall CVAD survival was similar in both groups. A limitation of this study were the small study groups. (46) Clark et al. performed a prospective cohort study investigating the TCL in pediatric patients (n=19) with oncologic and intestinal diseases. The CLABSI incidence rate decreased from 5.5 to 0.5 per 1,000 CVAD-days (RR: 0.09, p<0.01) with the use of TCL compared to the HL. The mean time to first CLABSI increased from 87 days to 296 days after TCL implementation (p=0.01). There were no episodes of hypocalcaemia observed during TCL implementation. A limitation of this study was the small study group. (49) Chong et al. performed a cross over prospective study investigating the TCL in pediatric oncology patients (n=20). The CLABSI incidence rate decreased from 14.4 to 2.4 per 1,000 CVAD-days (RR: 0.16, p<0.01) with the use of TCL compared to the HL. Two patients experienced central line occlusion for which one switched to the TCHL, one patient experienced nausea and vomiting after lock instillation. (50) All studies performed in pediatric oncology patients are summarized in Table 3. (45-50)

Table 3: Summary of studies performed in pediatric oncology patients (45-50)

Author (year)	Design (adult/pediatric)	Lock type (control – intervention)	Total number of patients or CVADs* (control - intervention	CVAD infections per 1,000 CVAD-days (control – intervention), Rate Ratio (RR), p-value	Number of infections (%/patients or %/cvads*) (control – intervention)	Reduction (control – intervention)	Adverse events
Handrup et al. (2013)	Open-labelled RCT	Heparin 250 IU/ml – taurolidine 1.35% / citrate 4% / heparin 100 IU/ml	112 (64 – 65)	1.4 – 0.4, RR: 0.28, p<0.01	26 (40.6) – 7 (10.8)	74%	Unpleasant taste in the majority of the patients.
Dumichen et al. (2012)	Open-labelled RCT	Heparin 100 IU/ml – taurolidine 1.35% / citrate 4%	71 (36 – 35)	1.3 -0.3, RR: 0.23 p=0.03	9 (25.0) - 2 (5.7)	77%	Taste sensations, nausea and vomiting, discomfort of chest and neck, perioral dysesthesia (n=7, 20%)
Chong et al. (2020)	Prospective cross over study	Heparin X IU/ml - taurolidine 1.35% / citrate 4%	33 (oncologic patients n=20 single arm)	14.4 – 2.4, RR: 0.16 p<0.01	X (X) – X (X)	X	Two patients experienced CVAD occlusion for which one patient switched to a TCHL. One patient experienced nausea and vomiting.
Clark et al. (2018)	Prospective cohort study	Heparin 10-100 IU/ml – taurolidine 1.35% / citrate 4%	19 (oncologic patients n=9 single arm)	5.5 – 0.5, RR: 0.09 p<0.01	39 (205.3) – 5 (26.3)	87%	No adverse events described
Simon et al. (2008)	Prospective cohort study	Heparin 200 IU/ml – taurolidine 1.35% / citrate 4%	179 (90 – 89)	All BSIs: 4.93 – 3.82, RR: 0.77 p=0.35 CoNS/MRSE infections: 2.3 – 0.45, RR: 0.20 p<0.01.	All BSIs: 30 (33.3) – 25 (28.1) CoNS/MRSE infections: 14 (15.5) – 3 (3.4)	All BSIs: 16% CoNS/MRSE infections: 78%	Unpleasant taste after flushing, pain during lock instillation in a peripheral catheter.
Ince et al. (2014)	Retrospective	Heparin 100 IU/ml – taurolidine 1.35% / citrate 4%	108 (33* – 79*)	X	16 (48.5)* – 18 (22.8)*, p=0.03	53%*	X

Evaluating the literature published on the different lock solutions, our hypothesis is that a lock solution containing taurolidine, citrate and heparin (TauroLock-Hep100™) is the most promising, safe and appropriate lock solution for pediatric oncology patients.

TauroLock-Hep100™

TauroLock-Hep100™ is a lock solution containing taurolidine 1.35%, citrate 4% and heparin 100 IU/ml. TauroLock-Hep100™ is produced by TauroPharm GmbH, Waldbuttelbrunn, Germany.

Taurolidine is metabolized into water, carbon dioxide, and the amino sulfonic acid taurine, which has an anti-biofilm activity and broad-spectrum antimicrobial activity against fungi (incl. *Candida albicans*), Gram-negative (incl. *Pseudomonas aeruginosa, Stenotrophomonas maltophilia*) and Gram-positive (incl. *Staphylococcus aureus*, coagulase negative

staphylococci*, and enterococci*) bacteria in vitro. (58-61) Taurolidine reduces adherence of bacteria to human epithelial cells and damages the cell walls of bacteria. In vitro, taurolidine even shows anticoagulant activities. (58-61) The major benefit of taurolidine is that in vitro, no evidence of microbial resistance against taurolidine has been found when tested against a broad spectrum of microorganisms. (59, 62) The most commonly described concentration of taurolidine in literature is 1.35% and does not show clinically relevant differences to taurolidine 2.0%. (9, 48, 58, 61, 62) This concentration is at least 10 times higher than the minimal inhibitory concentration $(MIC)_{50}$ for the majority of Gram-negative and Gram-positive microorganisms. (62) As described above, different lock solutions containing taurolidine are available. Olthof et al. tested the amount of microbial growth inhibition between different lock solutions containing taurolidine in vitro. They found minor differences in microbial growth inhibition and stated that these differences would not be relevant in the clinical setting. Furthermore, they found a decrease in thrombus weight due to taurolidine. This was, however, not as effective as citrate or heparin. Therefore, they advised that patients may benefit from the addition of heparin and/or citrate to taurolidine lock solutions. (61) High-dose concentrations of taurolidine (290 mg/kg, 2% taurolidine) have been associated with liver injury in mouse models. Low-dose concentrations of taurolidine (140 mg/kg, 2% taurolidine) which are similar to the TCHL dose did not show significant differences in liver injury compared to the control group (physiologic saline).(63) Lastly, hypersensitivity reactions to taurolidine are possible. (9, 18, 20, 45-48, 50)

Citrate has calcium-chelating properties, which results in both an anticoagulation and antimicrobial activity. (9, 64) Available solutions of citrate have concentrations ranging from 4 to 46%. Pittiruti et al. describes that higher concentrations of citrate are associated with a higher efficacy of CVAD-occlusion prevention. However, the European Renal Best Practice (ERBP), American Society of Diagnostic and Interventional Nephrology (ASDIN) and the Food and Drug Administration (FDA) advise to use a concentration of no more than 4% citrate in the prevention of central line related bloodstream infections (CRBSI), due to a case report of a patient that suffered cardiac arrest secondary to hypocalcaemia after injection of 46.7% citrate in the CVAD. (9) The described side-effects associated with the TCHL are presumably explained by spill-over/accidental flushes of citrate into the bloodstream. These side-effects include perioral dysesthesia, discomfort of neck and chest, dysgeusia, nausea and vomiting. (23, 34, 35, 37, 39, 40, 45, 46, 48, 50) All side effects are temporarily, described if the TCHL is instilled to fast, if the TCHL is accidentally flushed instead of aspirated and were only in rare occasions a reason to withdraw from the studies performed. Additionally, hypersensitivity reactions to citrate are possible. (9, 18, 20, 45-48, 50)

Heparin is a naturally occurring anticoagulant which prevents the coagulation of blood in-vivo and in-vitro. It potentiates the inhibition of several activated coagulation factors, including thrombin and factor X. Heparin prevents the progression of an obstruction by inhibiting further clot formation and allowing the activation of natural clot lysis. Heparin has a half-life of 1-2 hours. In haemodialysis patients the more frequent need for thrombolysis in patients receiving the TCL compared to the HL is described. (18, 20-22, 25, 26) This however, did not result in a higher frequency of CVAD removal in these patients. (18, 20) Solomon et al. advised to add 500 IU/ml heparin to the lock solution in haemodialysis patients. (20) In pediatric oncology patients, Handrup et al. used the TCL with the addition of 100 IU/ml heparin to prevent the CVAD from occlusions and CVAD-related CVTs. In this study, no CVADs were removed due to occlusion or thrombosis. (46) Due to the possible higher rate of occlusion due to blood clotting using the TCL, and similar rates of CVT/occlusion associated with the addition of heparin 100 IU/ml, we chose for the addition of heparin 100 IU/ml to the TCL. (18, 20) Side effects related to heparin, which are very rare,

are: hypersensitivity reactions, drug incompatibilities, and heparin-induced thrombocytopenia. In rare occasions, when the wrong dosage is used, iatrogenic hemorrhages can occur. (9, 65)

In this study, to avoid the above mentioned side-effects, the lock volumina are adjusted to the lumen of the CVAD that is inserted, the locks will be aspirated before instillation of a new lock, the locks will be instilled slowly (<1 ml per second), heparin is added to the solution for the prevention of the more frequent dispense of thrombolytics, and an ultrasound of the insertion veins to detect CVTs will be performed if CVT related symptoms are observed during the study.

Purpose of this study

Hypothetically, the TCHL will reduce the CLABSI rate compared to the HL. Additionally, the use of the TCHL may reduce the frequency of systemic antibiotic treatment, result in lower rates of CVAD-removal, fewer days of hospital/ICU admission, and a reduced mortality rate. Patients will benefit directly from reduced and more appropriate antibiotic use, which will also lead to a reduced risk of developing antibiotic resistance. Previous studies performed on the efficacy of the TCL or TCHL in pediatric oncology patients did not include enough patients to confirm the superior efficacy of the TCHL. Therefore, these studies do not deliver enough evidence to implement the TCHL in the pediatric oncology care in the Netherlands. (45-50) Due to the centralisation of the pediatric oncology care in the Netherlands, we are now able to include enough patients to finally draw a conclusion on the efficacy of the TCHL compared to the HL.

2. OBJECTIVES

Primary Objective:
To determine wheter the use of the taurolidine 1.35%, citrate 4%, and heparin 100 IU/ml lock solution (TauroLock™-Hep100) reduces the incidence of first tunneled central line associated bloodstream infections (CLABSI) compared to the heparin 100 IU/ml lock solution, in pediatric oncology patients, with a maximum follow-up of 90 days.

Secondary Objectives:
To compare the efficacy of the taurolidine 1.35%, citrate 4%, and heparin 100 IU/ml lock solution (TauroLock™-Hep100) to that of the heparine 100 IU/ml lock solution on the:

- Time to first tunneled CLABSI since the insertion of the CVAD
- CLABSI incidence per 1,000 CVAD-days
- Incidence of symptomatic CVTs
- Incidence of bacteremia
- Incidence of local infections
- Dispense of thrombolysis/systemic antibiotic treatment due to CLABSIs/CVTs
- Incidence of and reasons for CVAD-removal
- Cultured microorganisms causing CLABSIs
- Days of hospital admission due to CLABSIs/CVTs
- Safety of the TCHL/HL in terms of known side effects, severe adverse events (SAEs), intensive care unit admission, and mortality rate due to CLABSIs/CVTs

3. STUDY DESIGN

The CATERPILLAR study is designed as a mono-centre, investigator initiated, open labelled randomized controlled trial (RCT). Patients who receive their first CVAD or patients who receive a second, third, fourth etc. CVAD after a CVAD-free interval of more than 12 months, will be asked to participate in this study. These patients will be included in 29 months. Patients will be randomized into the HL study arm (n=231) or TCHL study arm (n=231). The lock will be instilled in the Princess Maxima Center with a maximum of once weekly (if admitted at the hospital or regulary visiting the hospital) and a minimum of once every three weeks (instillation before going home or to a different hospital for >1 week). In between, all patients will receive heparin 100 IU/ml. All patients will be followed up from CVAD insertion until the first CLABSI episode, CVAD-removal, second CVAD insertion (excl. stem cell apheresis CVADs) or death with a maximum study period of 90 days, whichever comes first. The maximum study period of 90 days was chosen since a great deal of the CLABSI episodes occur within the first 90 days after insertion. [Figure 2 and 3] (1, 2) All data (incl. shared care hospital data as this is standard of practice) will be collected in the Princess Máxima Center for Pediatric Oncology.

In the first months after diagnosis and CVAD insertion, patients will receive their oncologic treatment at the Princess Máxima Center for Pediatric Oncology. After one-two months, a minority of the patients will be treated in the shared care hospitals close to their homes and will return at least every three-six weeks to the Princess Máxima Center for Pediatric Oncology. Since the majority of the patients will be treated in the Princess Máxima Center in the first 90 days of their treatment (our follow-up time) we concluded that the benefits would not outweigh the expenses and difficult logistical execution of the instillation of the TCHL in all shared care hospitals in the Netherlands.

In consultation with the Trial Pharmacy of the University Medical Center Utrecht (UMCU) we chose for an open-labelled design since blinding of the lock ampoules would be too difficult and expensive since the design of the lock ampoules are not similar. Blinding with labels would not be sufficient. At first, we tried to find pharmacies that could fabricate similar ampoules with Taurolock-Hep100. The fabrication of TauroLock-Hep100 ampoules would cost >4 million euro or a bulk solution should be sent from TauroPharm to the pharmacy, which is also very pricely, logistically difficult and unusual. Another option discussed was to pre-fill syringes by pharmacies or unblinded nurses. This would need to be done for the heparin and TauroLock-Hep100 solution since neither of them are commercially available in 3mL pre-filled syringes. If performed by unblinded nurses the locks will expire after 24 hours and if performed by pharmacies the locks will expire after 7 days. Therefore, this option would also have resulted in high costs and would logistically be difficult to execute. Therefore, we concluded that the advantages did not outweigh the high costs and logistically difficult execution of a double-blinded RCT. Additionally, we formed an expert panel of three blinded specialists (microbiologists and infectiologists) to evaluate all positive bloodcultures and score them as CVAD associated or not.

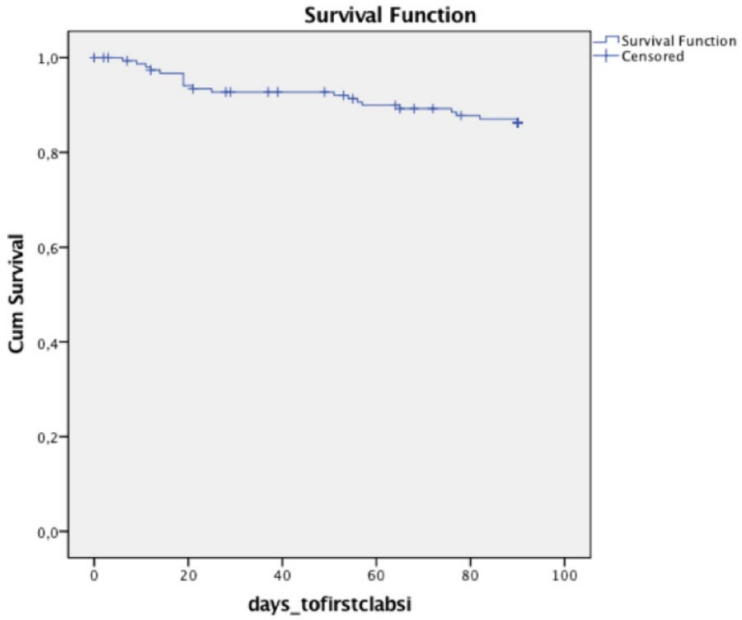


Figure 2: Kaplan-Meier curve of the first 90 days of insertion based on the data from the retrospective study performed by van den Bosch et al. (2019)(1) On the x-axis the days to first observed CLABSI per patient since the insertion of the CVAD. On the y-axis the cumulative CLABSI free survival. A CLABSI in the first 90 days was observed in 12.8% of the patients that received a CVAD.

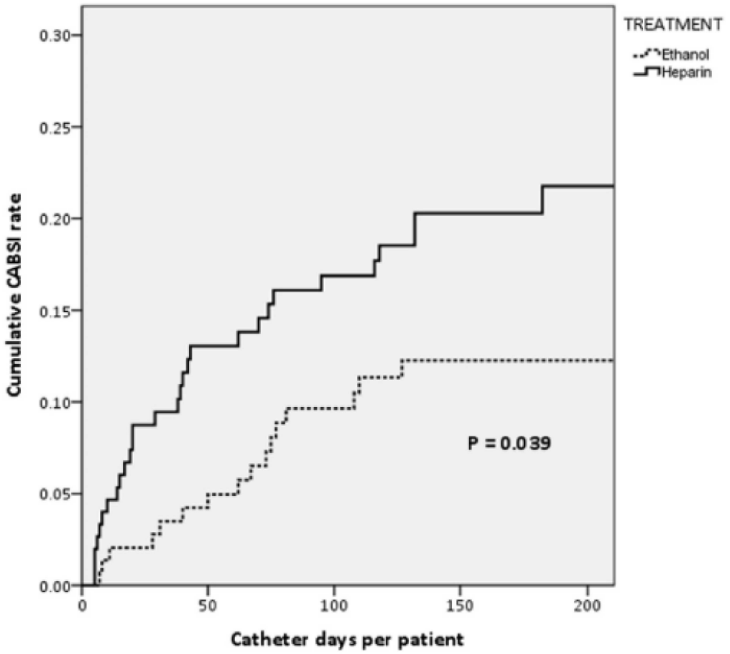


Figure 3: Kaplan-Meier curve of the first 200 days of insertion based on the data from the randomized controlled trial performed by Schoot et al. (2015) (2) On the x-axis the CVAD days to the first observed CLABSI per patient since the insertion of the CVAD. On the y-axis the cumulative CLABSI rate.

4. STUDY POPULATION

4.1 Population

All consecutive pediatric oncology patients (hematologic, solid and neurologic malignancies), treated in the Princess Máxima Center for Pediatric Oncology, ranging from 0-19 years old, receiving a tunnelled CVAD (H-CVAD/PL or TIVAP) for the first time or if their previous CVAD has been removed >12 months ago, will be asked to participate in this study. From May 2018, all pediatric oncology patients in the Netherlands are treated at the Princess Máxima Center for Pediatric Oncology. We expect that the planned number of patients can be recruited in 29 months from the defined source population.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Age between 0 - <19 years
- Radiological, cytological or histological proven paediatric malignancy (hematologic, solid, and neurologic malignancies)
- H-CVAD/PL or TIVAP to be inserted at the Princess Máxima Center for Pediatric Oncology
- Planned CVAD insertion of >90 days
- Written consent signed according to local law and regulations
- Parents/guardians or patient are willing and able to comply with the trial procedure

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- A previous CVAD removed < 12 months ago.
- Expected treatment for a majority of the follow-up time in a different hospital than the Princess Maxima Center for pediatric oncology in the first 90 days of inclusion resulting in difficulties/the inability to visit the Princess Maxima Center at least once every 3 weeks.
- Primary immunological disorder
- Contra indications: known hypersensitivity to taurolidine, citrate or heparin, and a history of heparin-induced thrombocytopenia.
- Documented bacteremia in the period from 24h before catheter insertion until inclusion
- Insertion of the CVAD at the same site as a previously confirmed CVT
- Pregnant, not willing to use adequate contraceptives, or breast-feeding

4.4 Sample size calculation

Our own database of CVAD associated complications (2015-2017) showed that 12.8% of the patients with an H-CVAD/PL or TIVAP developed at least one CLABSI within 90 days after insertion of their first CVAD (or second/third/etc. CVAD if their previous CVAD was removed >12 months ago). (1)

Group sample sizes of 206 in the TCHL-group and 206 in the HL-group achieve 80% power to detect a difference between the group proportions of 0.0780. The proportion in the TCHL-group (the treatment group) is assumed to be 0.1280 under the null hypothesis and 0.050 under the alternative hypothesis. The proportion in the HL-group (the control group) is 0.1280. The statistic test used is the two-sided Z-Test with unpooled variance.

An interim analysis will be performed after the inclusion of 231 patients. The level of test for the final analysis must be adjusted since part of the alpha will be used in the interim analysis. The level is based on the following computations. The first quantile (for the interim analysis) is set in such a way that the two-sided probability P(|U1| > q1) = 0.01 where U1 is the test used at the interim analysis and P means probability. For the law of large numbers U1 has a normal distribution with mean 0 and variance 1. This implies that the first quantile for the interim analysis is equal to 2, 575829. To compute the second quantile the joint distribution (U1, U2), which is bivariate normal with variances 1 and correlation 1/ √2 need to be employed. The second quantile needs to satisfy P(|U1| > q1 or |U2| > q2) = 0,05, or equivalently, P(-q1 < U1 < q1, -q2 < U2 < q2) = 0,95. The second quantile coming from the bivariate joint normal distribution (U1, U2) is equal to 2,002732; the corresponding nominal alpha level for the final analysis is therefore equal to 0.04520606.(66-71)

For each patient that prematurely drops-out of the study an extra patient will be included, we estimated that an extra 50 patients would be needed to account for potential drop-outs. The drop-out inflated sample size was therefore eventually calculated as 462 patients, 231 in each group. Our hypothesis is that the drop-out risk is minimal since all patients are seen regularly in the Prinses Maxima Center for pediatric oncology in the first 90 days of their treatment and the side-effects of the TCHL are minor and rare. The intention to treat principle is used in this study, therefore all patients are included in the final statistical analyses.

Since May 2018 all pediatric oncology patients are diagnosed and treated at the Princess Máxima Center, 550 new patients each year. Approximately 402 (73%) of these patients will receive a CVAD. (4) During the ARISTOCATHS-study, a similar study in the Netherlands investigating the ethanol lock in pediatric oncology children, 728 patients were screened for enrolment in the study, of which 421 (58%) patients were ineligible or declined to participate in the study. (2) In contrast to the ARISTOCATHS-study, during this study, all patients will be included in one center instead of eight and the TCHL is not associated with side effects like the ones associated with the ethanol lcok. Therefore, we hypothesized that 40% of the patients will be excluded or refuse to participate. Therefore, we hypothesized that we are able to include 240 patients each year (20 patients each month). To reach the total number of 462 patients, it will take us approximately 23 months. However, due to the risk of slow accrual, we added six months extra to the inclusion timeframe. Therefore, we estimate that it will take 29 months to include all patients. The last included patient will be followed-up for a maximum of 90 days, therefore the total study duration will be approximately 32 months. [Table 4]

Table 4: Planned study schedule

Months after start inclusion	What?	Description
0	Start inclusion	Planned start of the study
14.5	Interim database lock and interim analysis	After the inclusion of 50% of the patients
29	Stop inclusion	After the inclusion of 462 patients
32	Stop follow-up	After a period of 3 months after the inclusion of the last patient
32 - 36	Database lock, statistical analysis, writing the clinical study reports, and drafting of the manuscript based on the clinical study reports.	From the stop of follow-up until manuscript submission.
36	Manuscript submission	Four months after the study has stopped.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Comparator study arm (HL-study arm): Patients participating in the HL-study arm will receive the current standard of care lock solution containing heparin 100 IU/ml. The HL will be instilled in the CVAD lumen once a week if the CVAD is disconnected for preferably multiple days (at least >1 hour) until the next treatment. If patients are going home, to a different hospital, or do not have to visit the Princess Máxima Center for >1 week the CVAD will be locked and the lock will be be replaced within 3 weeks if they visit the Princess Máxima Center for Pediatric Oncology. To summarize, the maximum amount of study lock instillations is once every week and the minimum is once every three weeks. The lock volume depends on the CVAD type. [Table 5] The HL will be aspirated from all lumina before instillation of a new lock.

Investigational study arm (TCHL-study arm): Patients participating in the TCHL-study arm will receive the current standard of care lock solution containing taurolidine 1.35%, citrate 4.0%, and heparin 100 IU/ml. The TCHL will be instilled in the CVAD lumen once a week if the CVAD is disconnected for preferably multiple days (at least >1 hour) until the next treatment. If patients are going home, to a different hospital, or do not have to visit the Princess Máxima Center for >1 week the CVAD will be locked and the lock will be replaced within 3 weeks if they visit the Princess Máxima Center for Pediatric Oncology. To summarize, the maximum amount of study lock instillations is once every week and the minimum is once every three weeks. The lock volume depends on the CVAD type. [Table 5] The TCHL will be aspirated from all lumina before instillation of a new lock.

In between the above stated locking moments, the CVADs will be locked with standard heparin 100 IU/ml following the standard protocol of the Princess Máxima Center for Pediatric Oncology, home care institutions and all other shared care centers in the Netherlands.

5.2 Use of co-intervention

All co-interventions can be used as in usual clinical practice.

5.3 Escape medication

All escape medication can be used as in usual clinical practice.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Comparator study arm (HL-study arm)

Patients participating in the HL-study arm will receive the current standard of care lock solution containing heparin 100 IU/ml, 2 ml. The heparin lock will be aspirated before instillation of a new lock solution. The heparin 100 IU/ml lock is the standard of care in the Netherlands for locking CVADs. There is no registered heparin lock product available in the Netherlands. In the Princess Maxima Centre heparin 100 IU/ml, 50 ml is obtained via a so called "collegial delivery of pharmacy compounded medicinal products" (Dutch: "collegiaal doorgeleverde bereiding") This is an exception of The Dutch Medicines Act (www.igj.nl/zorgsectoren/geneesmiddelen-zonder-handelsvergunning/collegiaal-doorleveren). Heparin 100 IU/ml, 50 ml (ZI-number: 16037332) is produced by the Scheldezoom pharmacy (Spoorstraat 16, 4431 NK, 's-Gravenpolder, the Netherlands, https://www.scheldezoom.nl/algemeen). The Scheldezoom pharmacy is a GMP compounding pharmacy for expertise, preservation, and nation-wide delivery of commercially unavailable but rationally necessary medicines (GMP Report submitted in D2. of this METC submission). This product i.e. heparin 100 IU/mL, 50 ml is subsequently used to produce the final product, heparin 100 IE/ml, 2 ml in syringe for patient care. This final product is manufactured by the RIVA™ robot in the Pharmacy of the Prinses Maxima Center for Pediatric Oncology (Productdossier submitted in D2).

An officially registered comparable product is the BD PosiFlush™ Pre-filled Heparin Lock Flush. However, the BD PosiFlush™ Pre-filled Heparin Lock Flush is only registered in the United States of America (USA) and Canada. Therefore this product is not yet available in the Netherlands.(72, 73) The Food and Drug Administration (FDA) transferred the primary responsibility for the regulation of heparin catheter lock-flush solution products from the Center for Drug Evaluation and Research (CDER) to the Center for Devices and Radiological Health (CDRH). Heparin catheter lock-flush solution products are combined drug-device products. The transfer was based on the FDA's determination that the primary mode of action of these heparin catheter lock-flush solution products is that of the device part of the combination. (74) The BD PosiFlush™ Pre-filled Heparin Lock Flush is therefore registered as a medical device in the USA and Canada. (72, 73)

Investigational study arm (TCHL-study arm)

Patients participating in the TCHL-study arm will receive a lock solution containing taurolidine 1.35%, citrate 4.0%, and heparin 100 IU/ml (TauroLock™-Hep100). TauroLock™-Hep100 is produced by TauroPharm GmbH, August-Bebel-Straße 51, D-97297, Waldbüttelbrunn (www.taurolock.com). TauroLock™-Hep100 is CE-accredited and registered as a class III medical device. TauroLock™-Hep100 is used in the authorised form for the authorised indication. The certificates, declaration of conformity, and instructions for use can be found in appendix 2. [Appendix 2]

6.2 Summary of findings from non-clinical studies

Comparator study arm (HL-study arm)

There are no non-clinical data of relevance which are additional to the information already included in the other paragraphs.

Investigational study arm (TCHL-study arm)

As described in more detail in the introduction and rationale, in vitro studies show that the TCHL has anti-coagulant, anti-biofilm, and antimicrobial activities, without evidence of

antibiotic resistance to taurolidine. (58-61, 75) Taurolidine has shown a broad-spectrum activity against fungi, Gram-positive and Gram-negative bacteria in vitro. (58-61) High-dose concentrations of taurolidine (290 mg/kg, 2% taurolidine) have been associated with liver injury in mouse models. Low-dose concentrations of taurolidine (140 mg/kg, 2% taurolidine) which are similar to the TCHL dose did not show significant differences compared to the control group (physiologic saline). (63) It was advised by Olthof et al. to add citrate and/or heparin to the lock solution with taurolidine to prevent the CVAD from occlusion. (61)

6.3 Summary of findings from clinical studies

Comparator study arm (HL-study arm)

The HL is the standard of care in the Netherlands to lock CVADs in children and adults. Heparin is a naturally occurring anticoagulant which prevents the coagulation of blood in-vivo and in-vitro. (9, 65) Multiple studies have been performed to compare the efficacy of heparin to saline in the prevention of CVAD occlusion. The majority of these reports failed to show a superiority of heparin. (9)

Investigational study arm (TCHL-study arm)

A detailed description of the results and limitations of all clinical studies found in literature on the efficacy of the TCHL is to be found in the introduction section; below you will find a brief summary. The use of the TCL/TCHL showed decreased incidence rates of infections related to the CVAD in haemodialysis patients, total parenteral nutrition patients, and oncology patients compared to lock solutions containing saline or heparin. (12-41, 43, 45-50) Six studies have been performed in pediatric oncology patients. (45-50) Simon et al. performed a prospective cohort study (n = 179) and showed a significant decrease in infections due to CoNS and MRSA in the TCL study arm compared to the HL study arm (0.45 vs. 2.30 per 1,000 CVAD-days, $p<0.01$), however no difference in the incidence rate of bacteraemia was found between the two study arms. (48) Dumichen et al. performed an open labelled RCT (n = 71) and found a significant decrease in the incidence rate of bacteraemia in the TCL study arm compared to the HL study arm (1.30 vs. 0.30 per CVAD-days, $p=0.03$). (45) Ince et al. performed a retrospective study (n = 108) and showed a decrease in the CLABSI rate (48.5% vs. 22.8%, $p=0.03$), an increased duration of CVAD use, and a lower rate of catheter removal in the TCL study arm. (47) Handrup et al. performed the only open labelled RCT (n = 112) to compare the HL with the TCHL in pediatric oncology patients. They found a decrease in the incidence rate of CLABSI (1.40 vs. 0.40 per 1,000 CVAD-days, $p<0.01$), an increased time to CLABSI, and a reduction of fungi, Gram-positive and Gram-negative microorganisms in the TCHL study arm. Especially, CLABSIs caused by CoNS were reduced by 66% in the TCHL group. The incidence of removal due to occlusion and CVT, and overall CVAD survival were similar in both groups. (46) Recently, Clark et al. performed a prospective cohort study investigating the TCL in pediatric patients (n=19) with oncologic and intestinal diseases. The CLABSI incidence rate decreased from 5.5 to 0.5 per 1,000 CVAD-days ($p<0.01$) with the use of TCL compared to the HL. The mean time to first CLABSI increased from 87 days to 296 days after TCL implementation ($p=0.01$). There were no episodes of hypocalcaemia observed during TCL implementation. (49) Chong et al. performed a cross over prospective study investigating the TCL in pediatric oncology patients (n=20). The CLABSI incidence rate decreased from 14.4 to 2.4 per 1,000 CVAD-days ($p<0.01$) with the use of TCL compared to the HL. Two patients experienced central line occlusion for which one switched to the TCHL, one patient experienced nausea and vomiting. (50) All studies performed in pediatric oncology patients only contained small study groups (n= ≤ 180) and were therefore not considered as enough evidence to implement the TCHL in the pediatric oncology care in the Netherlands. (45-50)

6.4 Summary of known and potential risks and benefits

Comparator study arm (HL-study arm)

Heparin is a naturally occurring anticoagulant which prevents the coagulation of blood in-vivo and in-vitro. It potentiates the inhibition of several activated coagulation factors, including thrombin and factor X. Heparin prevents the progression of an obstruction by inhibiting further clot formation and allowing the activation of natural clot lysis. Heparin has a half-life of 1-2 hours when it enters the bloodstream. Used as directed, it is extremely unlikely that the low levels of heparin reaching the blood will have any systemic effect. However, if the heparin does reaches the bloodstream possible side effect can occur: hypersensitivity reactions, heparin-induced thrombocytopenia and drug incompatibilities. In extremely rare occasions, when the wrong dosage is used, iatrogenic hemorrhages can occur. (9, 65)

Investigational study arm (TCHL-study arm)

A detailed description of the risks and benefits of the TCHL is to be found in the introduction section; below you will find a brief summary. Hypothetically, the TCHL will reduce the CLABSI rate. Therefore, the TCHL may reduce the administration of antibiotics, result in lower rates of CVAD removal, fewer days of hospital/ICU admission, and a lower mortality rate due to CLABSI. Patients will benefit directly from reduced and more appropriate antibiotic use, without the risk of antibiotic resistance development. (12-41, 43-50)

The expected side effects are temporarily, caused by a spill-over of citrate, and only described if the TCHL is instilled to fast or if the TCHL is accidentally flushed instead of aspirated: perioral dysesthesia, discomfort of neck and chest, dygeusia, nausea and vomiting. (23, 34, 35, 37, 39, 40, 45, 46, 48, 50) Hypocalcaemia events causing arrhythmias have only been associated with much higher concentrations of citrate, which are not used in this study. Additionally, hypersensitivity reactions, and heparin induced thrombocytopenia are possible side effects, but in literature only one patient has been described in whom an anaphylactic-like reaction was observed. (34) Liver-injury is associated with high-concentrations of systemic taurolidine in mouse-models, the TCHL contains low-dose taurolidine, which is not associated with liver-injury. A more frequent dispense of thrombolytics has been associated with lock solutions containing taurolidine and citrate in haemodialysis patients. This was only observed without the addition of heparin. (18, 20-22, 25, 26) In this study, the lock volumina are adjusted to the lumen of the CVAD that is inserted, the locks will be aspirated before instillation of a new lock, the locks will be instilled slowly (<1 ml per second), heparin is added to the solution for the prevention of the more frequent dispense of thrombolytics, and an ultrasound of the insertion veins to detect CVTs will be performed if CVT related symptoms are observed during the study. If aspiration is not possible, TauroPharm suggests to apply the lock not faster than 1 ml per eight seconds. In this case only a total of ≤2.6ml of the lock solution will reach the bloodstream. (48) The citrate will dilute so fast that no problems concerning the calcium concentration are suspected. (46, 48, 64)

6.5 Description and justification of route of administration and dosage

Comparator study arm (HL-study arm)

Description

Patients participating in the HL-study arm will receive the current standard of care lock solution containing heparin 100 IU/ml. The HL will be instilled in the CVAD lumen once a week if the CVAD is disconnected for preferably multiple days (at least >1 hour) until the next treatment. If patients are going home, to a different hospital, or do not have to visit the

Princess Máxima Center for >1 week the CVAD will be locked and the lock will be be replaced within 3 weeks if they visit the Princess Máxima Center for Pediatric Oncology. The lock volume depends on the CVAD type. [Table 5] The HL will be aspirated before instillation of a new lock. In between, all patients will be locked with heparin 100 IU/ml following the CVAD-manipulation protocol of the Princess Máxima Center for Pediatric Oncology and all shared care hospitals in the Netherlands. (9, 65, 76)

Justification
Guidelines recommend the use of heparin at 10-100 IU/ml for CVAD locking, 10 IU/ml for daily flushing and 100 IU/ml for periodic locking. In the Princess Máxima Center for Pediatric Oncology and all shared care hospitals in the Netherlands we chose for 100 IU/ml since most CVADs are locked periodically. (9, 65, 76, 77) The lock frequency and aspiration of the lock will be performed in this group to make both investigational groups equal.

Investigational study arm (TCHL-study arm)
Description
Patients participating in the TCHL-study arm will receive a lock solution containing taurolidine 1.35%, citrate 4.0%, and heparin 100 IU/ml. The TCHL will be instilled in the CVAD lumen once a week if the CVAD is disconnected for preferably multiple days (at least >1 hour) until the next treatment. If patients are going home, to a different hospital, or do not have to visit the Princess Máxima Center for >1 week the CVAD will be locked and the lock will be replaced within 3 weeks if they visit the Princess Máxima Center for Pediatric Oncology. The lock volume depends on the CVAD type. [Table 5] The TCHL will be aspirated before instillation of a new lock. In between, all patients will be locked with heparin 100 IU/ml following the CVAD-manipulation protocol of the Princess Máxima Center for Pediatric Oncology and all shared care hospitals in the Netherlands. (9, 65, 76)

Justification lock dosage
Available solutions of citrate have concentrations ranging from 4 to 46%. Pittiruti et al. describes that higher concentrations of citrate are associated with a higher efficacy of CVAD-occlusion prevention. However, the European Renal Best Practice (ERBP), American Society of Diagnostic and Interventional Nephrology (ASDIN) and the Food and Drug Administration (FDA) advise to use a concentration of no more than 4% citrate in the prevention of central line related bloodstream infections (CRBSI), due to a case report of a patient that suffered cardiac arrest secondary to hypocalcaemia after injection of 46.7% citrate in the CVAD. (9, 18, 20, 45-48, 50) In order to prevent the above stated side effects we will use citrate 4.0%, we adjusted the lock volumes to the lumen of the CVADs, and we will aspirate the lock before use of the CVAD. If aspiration, on rare occasions, is not possible, TauroPharm suggests applying the lock not faster than 1 ml per eight seconds. If this happens only a maximum total of ≤2.6ml of the lock solution will reach the bloodstream. (48) The citrate will dilute so fast that no problems concerning the calcium concentration are suspected. (64)

Concentrations of 1.35% and 2.0% taurolidine are described in literature, no clinically relevant differences were found between the two concentrations. (9, 48, 58, 61, 62) These concentrations are at least 10 times higher than the MIC_{50} of the majority of Gram-negative and Gram-positive microorganisms. (62) A concentration of 1.35% taurolidine is the most commonly used in pediatric oncology patients. (9, 48) The microbial destruction time of taurolidine in vitro is 30 minutes, therefore the TCHL needs to be in situ for at least >1 hour. (78, 79)

In pediatric oncology patients, heparin 100 IU/ml is added to the TCL, in comparison with the addition of 500 IU/ml heparin, which is used in haemodialysis patients. The TCHL is associated with equal removal rates due to CVT compared to the HL alone in pediatric oncology patients. (46, 64) To further prevent the CVAD from occlusion, proper flushing policies, needle free connectors and no-reflux strategies are used during the administration of the lock solution. (9) Heparin 100 IU/ml is also the preferred dosis for the standard of care heparin lock. (9, 65, 76)

Justification of volume
Literature advices to minimize the lock volume to minimize leakage into the bloodstream. The minimum volume is the volume of the CVAD, since the CVAD lumen has to be filled entirely. During insertion the CVADs will be trimmed to fit the individual child, therefore the volume will be less than the company's stated CVAD priming volume (a difference of 0.02-0.20 ml per 10 cm). The true volumes of the CVAD and the advised lock volumes can be found in table 5. [Table 5] If the positive pressure technique is performed inadequetly, it is possible that a small volume is not injected into the CVAD, therefore all lock volumes are 15-20% higher than the maximal catheter volume (as adviced in literature). (65, 67) The CVAD volume includes the catheter, huber needle with wire (0.3 ml), three-way valve (0.2 ml), and needle-free connector (Clave®) (0.05 ml).

Table 5: Lock Volumina

CVAD	Type	Diameter (Fr)	Maximal catheter volume (ml)	Lock volume (ml)
TIVAP	Babyport®	4.5	0.80	1.0
	Low-profile®	6.5	1.04	1.5
	Standard®	6.5	1.28	1.5
Broviac®	Single lumen	6.6	0.74	1.0
Hickman®	Double lumen	7.0	0.90/0.80	1.0/1.0
Powerline®	Double lumen	6.0	0.70/0.70	1.0/1.0
	Triple lumen	6.0	0.75/0.62/0.62	1.0/0.8/0.8

Justification lock frequency
The instructions for use of TauroLock-Hep100 do not give an advice about the maximum amount of locks that can be instilled in a certain time frame. The instructions only state: "TauroLock-Hep100 will remain inside the access device until the next treatment (for a maximum of 30 days)." The studies performed in pediatric oncology patients Schoot et al., Handrup et al., and Simon et al. all locked the CVAD mostly once and sometimes twice a week. All observed a significant reduction of the amount of CLABSIs. (2, 46, 48) Clark et al. locked the CVAD daily and Ince et al., Chong et al. and Dumichen et al. did not report their lock frequency. (47, 49, 50) Daily locks might be safe, however due to the minimal amount of evidence and the possible side effects associated with high concentrations of citrate, we decided to choose a maximum lock frequency of once a week similar to most performed pediatric oncology studies. (9, 18, 20, 45-48, 50)

We chose for a minimum lock frequency of at least once every three weeks if patients are not seen at the Princess Máxima Center for >1 week so that these patients do not have to travel to the Princess Máxima Center every week only for the study lock. We chose specifically for three weeks since most patients are at least seen once every three weeks at our hospital and the TCHL can remain in situ for a maximum of 30 days. We did not choose for a minimum frequency of >3 weeks since it is possible that in between the lock is removed by

home care or shared care nurses. This way we can ensure that every patient has a lock in situ at least once every three weeks.

6.6 Dosages, dosage modifications and method of administration

Dosages

Lock volume depends on the CVAD type [Table 5]. A minimum of 5 and maximum of 13 locks per patient will be instilled in the follow-up of 90 days.

- TCHL-study arm: taurolidine 1.35%, citrate 4% and heparin 100 IU/ml.
- HL-study arm: 100 IU/ml heparin.

Method of administration

Five steps of administration (48):

1. Flush the device with 10 mL of saline.
2. Withdraw the lock from the vial/ampoule using an appropriate syringe.
3. Instill the lock slowly (not more than 1 mL per second, infants and children less than two years of age not more than 1 mL per 5 seconds) into the access device in a quantity sufficient to fill the lumen completely. [Table 5] The lock will remain inside the access device until the next treatment (for a maximum of three weeks).
4. Prior to the next treatment, the lock must be aspirated from all lumina and discarded. In the advent of inability to aspirate from the device, the lock should be flushed very slowely <1 mL/5 sec.
5. Flush the device with 10 mL of saline.

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable, since this study is submitted as a medical device study, see paragraph 6.1.

6.8 Drug accountability

Shipment and receipt

The TCHL will be shipped from the TauroPharm GmbH (Waldbüttelbrunn, Germany) to the clinical trial pharmacy of the Princess Máxima Center for Pediatric Oncology. The HL that will be given as a study lock in the Princess Máxima Center for Pediatric Oncology will be shipped from the Scheldezoom pharmacy (‘s-Gravenpolder, the Netherlands) to the clinical trial pharmacy of the Princess Máxima Center for Pediatric Oncology.

Disposition

After inclusion the physician will register an order (VMO) in the patient file in Chipsoft EZIS/HiX for the randomized lock, either the TCHL or HL. The nurses will recognize in which study arm the patient is randomized by the CATERPILLAR patient-card. Additionally, the nurses can check the order (VMO) for either the TCHL or HL that is registered in the patient file in Chipsoft EZIS/HiX. The research nurses need to double check the lock solution (two signatures need to be written on the “Lock Instillation Form”) and register the batch number or stick the flag label on a paper “Lock Instillation Form” before instillation.

Return

All left over investigational products will return to the Trial Pharmacy of the Princess Máxima Center for Pediatric Oncology and be stored for later use after the study is performed.

Destruction

Expired investigational products will be destructed.

7. METHODS

7.1 Study parameters/endpoints

7.1.1 Main study parameter

Incidence of first tunneled CLABSIs since the insertion of the CVAD. All data-points that are needed for the evaluation of the occurrence of a CLABSI will be collected by the local data-manager. Three experts will blindly and independently judge if a CLABSI or no-CLABSI occurred in all patient based on the collected data and the CLABSI definition described in paragraph 7.1.5. All non unanimous judgements will be discussed between the experts until they all agree. If the experts still disagree, the final judgement will be based on the judgement of the majority.

7.1.2 Secondary study parameters

- Time to first CLABSI since insertion of the CVAD
- CLABSI incidence per 1,000 CVAD-days
- Incidence of symptomatic CVTs
- Incidence of bacteremia
- Incidence of local infections
- Dispense of thrombolysis/systemic antibiotic treatment due to CLABSI or CVT
- Incidence of and reasons for CVAD-removal
- Cultured microorganisms causing CLABSI
- Days of hospital admission due to CLABSI/CVT
- Safety of the TCHL/HL in terms of known side effects, SAEs, intensive care unit admission, and death due to CLABSI/CVT

7.1.3 Endpoints

Endpoints of the study are the first tunneled CLABSI episode (diagnosed by the expert panel), removal of the CVAD, second CVAD insertion (excl. stem cell apheresis CVADs) or death of the patient, whatever endpoint will come first with a maximum study period of 90 days. If an endpoint is reached, no more study locks will be given. The data of the patients will be followed-up until one month after the endpoint was reached.

7.1.4 Other study parameters

Patient characteristics and CVAD insertion:

- Age
- Gender
- Oncologic diagnosis
- Chemotherapy protocol and treatment arm
- Planned administration of prophylactic systemic antibiotics (trimethoprim/sulfamethoxazole = bactrimel®, ciprofloxacin, or anti-mycotics)
- Date of CVAD surgery
- Type of CVAD
- Introduction method (percutaneous/open)
- Lumen amount
- Lumen diameter
- Access vein and side

- Complicated procedure

Lock characteristics:
- During and directly after study lock instillation:
  - Date of lock instillation
  - Type of lock
  - Side effects (Adverse Device Events (ADE) of interest) and grade (CTCAE version 5.0, November 27, 2017)
- Aspiration of the study lock:
  - Date of removal
  - Inadvert lock removal at home
  - Lock aspirated, accidentally flushed, or malfunction
  - In case of malfunction: type of treatment
  - Side effects (Adverse Device Events (ADE) of interest) and grade (CTCAE version 5.0, November 27, 2017)

Suspicion of CLABSI characteristics:
- Start date of episode
- Presence of symptoms
- Is the CVAD inserted for >48 hours
- Allogenic stem cell recipient with diarrhea >1L in 24 hours, or allogenic stem cell recipient with graft versus host disease grade III or IV.
- Neutropenia episode (incl. duration and severity of neutropenia)
- Results of blood cultures (each lumen counts as one separate blood culture): date and microorganisms cultured will be registered.
- Other documented infection at the time of CLABSI with the same pathogen cultured as the blood culture
- CLABSI, MBI-LCBI, BSI, or suspicion CVAD-related infection without a positive bloodculture.
- In case of BSI, the reason why a BSI was scored e.g. not enough blood cultures, no symptoms, contamination, CVAD in situ for <48 hours, infection at a different site with same pathogen.
- Treatment
- Hospital admission and hospital admission days
- Intensive care unit admission and intensive care unit admission days
- Death of the patient

Suspicion of local infection characteristics:
- Start date of episode
- Symptoms
- Results of blood cultures
- Treatment
- Hospital admission and hospital admission days
- Intensive care unit admission and intensive care unit admission days
- Death of the patient

Suspicion of CVT characteristics:
- Date start episode
- Type of symptoms possibly related to a CVT
- Signs of CVT on radiological imaging
- Location thrombus

- Treatment
- Hospital admission days due to CVT
- Intensive care unit admission days due to CVT
- Death of the patient due to CVT

Serious Adverse Device Events (SADEs)
- ADE term (80)
- Start date ADE
- Date ADE turned into SADE
- Category of SADE
- SADE severity (toxicity grade) (80)
- Hospital admission date
- Medical intervention date
- Date SADE was resolved
- Description SADE
- Date of last lock administration and lock dosis
- Relationship of SADE to intervention (possible/definitely)
- Action taken
- Relevant medical history
- Relevant tests performed
- Study intervention discontinued due to the event

End of the study
- Reason end of the protocol
- In case of CVAD removal: reason, date, and catheter tip microorganisms culture
- In case of death of the patient: reason, date

7.1.5 Definitions

Bloodstream infection (BSI)	Every positive blood culture that is impossible to classify as a CLABSI or MBI-LCBI. Reasons why a BSI is scored: only one bloodculture with a common commensal is obtained, two bloodcultures are obtained but ≤2 common commensals or none recognized pathogens are cultured, positive blood cultures without observed symptoms (e.g. fever, chills, or hypotension, for patients <1 year: fever, bradycardia, and apnea), or an infection at another site with the same cultured pathogen is observed.
Chills	Chills described by parents and/or patient or witnessed by a physician.
Central-line associated bloodstream infection (CLABSI)	CLABSI will be scored if the patient meets one of the following criteria: (1) the patient has a recognized pathogen (micro-organisms not registered in the “List of Common Commensales” of the Centers for Disease Control and Prevention) cultured from ≥1 blood cultures, (2) the patient has at least one of the following signs: fever, chills, or hypotension (for patients <1 year: fever, bradycardia, and apnea), AND the same matching potential common 35ommensals (“List of Common Commensales” of the Centers for Disease Control and Prevention) are cultured from ≥2 blood cultures drawn on separate occasions (incl. two blood

	cultures drawn at the same time but from different lumen). Additionally, a CLABSI will only be scored if the CVAD is in situ for >48 hours on the date of the event, if the pathogen cultured is not related to an infection at another site AND if the MBI-LCBI criteria are not met. See appendix 3 for the CLABSI flow-chart. (67, 81)
Local infection (i.e. phlebitis, exit-site or tunnel-infections)	Positive exit-site culture, erythema, purulent drainage or tenderness within 2 cm of the CVAD track and exit-site
Central venous thrombosis (CVT)	If the patient has (1) peripheral veins that have a non-compressible segment, or (2) there is an echogenic intra-luminal thrombus or an absence of flow in the central venous system. (76)
Diarrhea	≥1L Diarrhea in a 24-hour period
Fever	Temperature >38.0°C on two occasions within a 12-hour period, one temperature >38.5°C, or one temperature <35.0°C (for patients of <1 year <36.0°C).
Hypotension	Hypotension criteria per age: ○ 0-3 Months: systolic RR<60 mmHg ○ 3 Months – one years: systolic RR<80 mmHg ○ 1-11 Years: systolic RR <90 mmHg ○ >12 Years: systolic RR<100 mmHg
Malfunction	If it is impossible to aspirate or flush the CVAD.
Mucosal barrier injury laboratory confirmed bloodstream infections (MBI-LCBI)	The mucosal barrier injury laboratory confirmed bloodstream infections (MBI-LCBI) were scored following the criteria of the CDC to exclude BSIs that are possibly the result of the weakened mucosal barrier of the gut in immunocompromised patients, and probably not associated with the CVAD. MBI-LCBI will be scored if: (1) a CLABSI with a recognized pathogen is scored AND the only pathogens cultured are intestinal organisms (micro-organisms registered as MBI Organisms in the "List of Common Commensales", CDC), OR (2) a CLABSI with two or more common commensals is scored AND the commensals cultured are only viridans streptococci. Additionally, the patients must meet one of the following during same hospitalization as the positive blood specimen: (1) the patient is an allogenic stem cell transplant recipient in the past year with grade III or IV gastrointestinal graft versus host disease, or > 1 litre diarrhea in a 24-hour period, OR (2) the patient is neutropenic on two separate days. See appendix 3 for the CLABSI flow-chart. (3, 67, 81-84)
Mild neutropenia	Granulocytes 1000-1500 x 10^6 /L
Moderate neutropenia	Granulocytes 500-1000 x 10^6 /L
Severe neutropenia	Granulocytes < 500 x 10^6 /L

Very severe neutropenia	Granulocytes < 100 x 10^6 /L

7.2 Randomisation, blinding and treatment allocation

Patients will be randomized between two treatment arms: HL- and TCHL-study arm. Randomisation will be done with the method of minimisation. Stratification will be done according to two factors: used type of CVAD (TIVAP or H-CVAD/PL) and diagnosis of cancer (hematologic or solid/neurologic malignancies).

The randomization will be done with the use of an online randomization service by internet (Software as a Service – SaaS) called ALEA®. This web-based randomization program will provide 24 hours 7 days per week service. At the study site, the researcher or research nurse will enter the randomization data in ALEA®. Notification will be sent to the local study team. The local study team will receive a notification with patient identifier, patient study number and the allocated treatment.

7.3 Study procedures

Information to patients

If it is determined that a patient will need a tunneled CVAD, the surgeon/researcher will inform the patient and parents/legal guardian about the CVAD insertion procedure. At the end of this conversation verbal information and information in writing about this study will be given to the patients and parents/legal guardian.

Inclusion

Inclusion (including first lock instillation) should take place within one week after CVAD insertion. However, if this is not possible due to clinical circumstances (i.e. physical and/or psychological) patients may be included (incl. first lock instillation) within four weeks after CVAD insertion. The researcher/research nurse will sign the informed consent papers after the patient and parents/legal guardian. The in- and exclusion criteria will be checked to determine if the patient is eligible for the CATERPILLAR-study. The researcher/research nurse will complete the inclusion details in HiX and will enter the patient information in the randomization programme ALEA®. The local data-manager will complete the “Registration and Baseline Form” in Castor EDC. Patients will be randomized in either the HL- or TCHL-group. The local study team will receive the randomization information. The surgeon/researcher registers an order (VMO) for either the TCHL or HL in the patient file in Chipsoft EZIS/HiX. See appendix 4 for the flow-chart of the study procedure described above. [Appendix 4]

All patients will receive a CATERPILLAR card with “YES and NO stickers” from the research nurse/researcherThis card is used to alert health care providers that the patient is a participant in the CATERPILLAR-study and will show in which group the patient is assigned and what lock volume needs to be instilled. Parents and/or patients will be asked to show the CATERPILLAR-card and stickers each time they visit the hospital.

Lock instillation and aspiration

Directly after the insertion of the CVAD, a running intravenous infusion will be connected to the inserted CVAD. The first investigational lock solution will be instilled in the first week after insertion. However, if this is not possible due to clinical circumstances (i.e. physical and/or psychological) patients may receive the first lock within four weeks after CVAD insertion. The other study locks will be instilled in the CVAD lumen once a week if the CVAD is

disconnected for preferably multiple days (at least >1 hour) until the next treatment. If patients are going home, to a different hospital, or do not have to visit the Princess Máxima Center for >1 week the CVAD will be locked and the lock will be replaced within 3 weeks if they visit the Princess Máxima Center for Pediatric Oncology. The lock volume depends on the CVAD type. [Table 5] In between, all patients will be locked with heparin 100 IU/ml following the CVAD-manipulation protocol of the Princess Máxima Center for Pediatric Oncology, home care organizations and all shared care hospitals in the Netherlands.

The research nurse/researcher can use the CATERPILLAR patient-card and VMO in HiX to see in which study group the patient is randomized. The nurses will be asked to double check the ampoule before instillation, two signatures and the batch number need to be written on a paper “Lock Instillation Form”. After the instillation of the new study lock solution, the patients will be asked questions concerning the experience of side effects during the lock instillation. The paper “Lock Instillation Form” will be completed by the research nurse. The patients will receive a “Lock in situ YES” sticker with the lock instillation date, that will be attached to the CATERPILLAR-card. Patients and/or parents will be asked to show the card during every visit in a hospital.

If the CVAD is manipulated again, the “Lock in situ YES” sticker on the CATERPILLAR card will alert health care providers that the study lock is in situ and that the lock needs to be aspirated by the research nurse or researcher. If the lock aspiration takes place in the Princess Máxima Center for Pediatric Oncology, again questions concerning the experience of side effects during lock removal will be asked and the “Lock Instillation Form” will be completed. Then the “Lock in situ NO” sticker with the aspiration date and method of removal will be attached to the CATERPILLAR-card.

If the study lock is aspirated in a shared care center or home care setting the nurse will be asked to follow the guidelines on the CATERPILLAR card and stick the “Lock in situ NO” sticker on the CATERPILLAR card with the date and method of removal. The research nurses in the Princess Maxima Center will be asked to register the lock removal date on the “Lock Instillation Form” the next time the patient visits the Princess Maxima Center. If the lock removal date is missing the shared care center will be contacted. If in the shared care centers or at home a regular heparin lock is instilled after the CVAD is used, patients will not be excluded from the study.

The data-manager will enter the information of the “Lock Instillation Form” in the online database “Lock Instillation Form” in Castor EDC.

<u>Suspicion of an (local) infection or CVT in the Princess Máxima Center for Pediatric Oncology</u>

In case of symptoms possibly associated with an (local) infection or CVT (e.g. swelling/pain at the catheter site, face, neck, arm, or shoulder) parents will be asked to contact a nurse or physician from the beginning of the signs of infections. If the patient is seen in the Princess Máxima Center for Pediatric Oncology, the surgeons/pediatric oncologists will inform the research nurse/researcher. Standard of care diagnostic work-up and treatment will be performed. The research nurse/researcher will register all relevant details in Chipsoft EZIS/HiX. The research nurse/researcher will alert the local data-manager and he/she will complete the “Suspicion of a CLABSI”, “Suspicion of a local infection” or “Suspicion of a CVT” form in Castor EDC. Episodes of CLABSIs, local infection or CVTs will be monitored until the symptoms have resolved and the patient has recovered. See appendix 5 for the

flow-chart of the study procedures described above. [Appendix 5] If a blood culture is drawn from the CVAD and the TCHL or HL is still in situ, the first 2.0 mL has to be discarded.

Suspicion of an infection or CVT in the shared care hospitals

In case of any symptoms possibly associated with an (local) infection or CVT (e.g. swelling/pain at the catheter site, face, neck, arm, or shoulder) parents will be asked to contact a nurse or physician from the beginning of the signs of infections. It is the standard of care in the Netherlands to inform the Princess Máxima Center for Pediatric Oncology if a patient is seen in a shared care hospital due to treatment complications (e.g. CLABSI, local infection or CVT). The physicians in the shared care hospitals enter the complication data in Chipsoft EZIS/HiX of the Princess Máxima Center for Pediatric Oncology and/or will call the patients' physician in the Princess Máxima Center for Pediatric Oncology. The physician/nurse of the Princess Máxima Center will contact the research nurse/researcher who will register all details in Chipsoft EZIS/HiX. The research nurse/researcher will alert the local data-manager to complete the "Suspicion of a CLABSI", "Suspicion of a local infection" or "Suspicion of a CVT" form in Castor EDC. If information is missing, the shared care centers will be contacted. See appendix 6 for the flow-chart of the study procedures described above. [Appendix 6] If a blood culture is drawn from the CVAD and the HL or TCHL is still in situ, the first 2.0 mL has to be discarded.

End of the study

The patient will reach the end of the study in case of a CLABSI episode, CVAD-removal, second CVAD insertion (excl. stem cell apheresis CVADs) or death of the patient, with a maximum of 90 days. After one of the endpoints of the study has been reached, the research nurse/researcher will enter the end of the protocol details in HiX. The data-manager will complete the "End of the Protocol Form" in Castor EDC. See appendix 7 for the flow-chart of this procedure. [Appendix 7]

Division of tasks

The research nurse and researcher will perform the informed consent procedure, keep track of all patients, make appointments and collect data in HiX. The local data-manager will collect data from HiX and enter this data into Castor. Central data management will check data completeness. The statistical analysis (interim analysis and final analysis) will be performed by a statistician. The DSMB charter submission will be done by the local study team. The manuscript will be written by the researcher and the PI.

"Extra"-procedures

All procedures that subjects undergo are part of the standard medical treatment of the Princess Máxima Center for Pediatric Oncology, except for the following:

- Parents need to show the CATERPILLAR-card to the nurse/physician during every hospital visit.
- In the Princess Máxima Center for Pediatric Oncology every patient participating in the HL- or TCHL-study arm will be asked to answer questions concerning the side effects after each lock instillation and after study lock removal.
- If a blood culture is obtained from a patient and the HL or TCHL is in situ, the first 2.0 mL has to be discarded and the lock is aspirated instead of flushed before instillation of a new lock.
- If the study lock is removed in a shared care hospital or home care setting the nurse will be asked to follow the guidelines on the CATERPILLAR card and stick the "Lock in situ NO" sticker to the card with the aspiration date and reason for removal.

Diagnostic procedures or treatment of these patients will not be postponed due to participation in this study.

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time, for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1. Specific criteria for withdrawal

1. Admission of >3 weeks in a hospital outside the Netherlands or a non-participating shared care centre.
2. Hypersensitivity reaction after instillation of the TCHL solution.

7.5 Replacement of individual subjects after withdrawal

The intention to treat principle will be used. Therefore, patients will not be replaced after withdrawal.

7.6 Follow-up of subjects withdrawn from treatment

Subjects that object to further participate in the study will receive the standard of care locks containing heparin 100 IU/ml. Their electronic patient files will be reviewed until 30 days after the last lock instillation.

7.7 Premature termination of the study

The DSMB can advise the sponsor to terminate the study prematurely. The sponsor or METC can decide to terminate a study.

Premature termination criteria:

- If the interim analysis shows an earlier disturbance of equipoise, e.g. major superiority or inferiority of the TCHL. See interim analysis, chapter 10.3, for more details.
- If significantly more or less SAEs/SUSARs are reported in the TCHL-group. See interim analysis description for more details.
- Methodological inaccuracies
- If the conduct is not feasible because of logistics or subject recruitment

If it is decided to terminate the study earlier than indicated in the protocol, all patients and involved hospitals will be informed by the researcher. The study must be stopped immediately. The sponsor is required to report premature termination to the reviewing committee (METC) within 15 days after termination stating the reason for early termination.

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

8.2 ADEs and SADEs

8.2.1 Adverse device effects (ADEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational products. Only AEs of special interest with a possible or definite relationship (serious adverse device effects ADEs) with the investigational products will be registered. Registration of all AEs would lead to the registration of too many AEs in this patient group.
Registration will be performed according to the definitions of the Common Terminology Criteria for Adverse Events (CTCAE version 5.0, November 27, 2017), incl. severity grade.

ADEs of special interest that are registered:

- Oral dysesthesia: A disorder characterized by a burning or tingling sensation on the lips, tongue or entire mouth.
- Neck pain: A disorder characterized by a sensation of marked discomfort in the neck area.
- Chest wall pain: a disorder characterized by a sensation of marked discomfort in the chest wall
- Dysgeusia: A disorder characterized by abnormal sensual experience with the taste of foodstuffs; it can be related to a decrease in the sense of smell.
- Nausea: A disorder characterized by a queasy sensation and/or the urge to vomit.
- Vomiting: A disorder characterized by the reflexive act of ejecting the contents of the stomach through the mouth.
- Allergic reaction: A disorder characterized by an adverse local or general response from exposure to an allergen.
- Blood and lymphatic system disorders - Heparin induced thrombocytopenia: thrombocytopenia due to the administration of heparin.
- Other ADEs that have not been anticipated before.

The research nurse/researcher will ask the patients and/or parents if any of the above described ADEs occur and register them on the “Lock Registration Form”. All ADE’s will be registered in the Castor EDC database by the local data-manager.

8.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or affect that

- Results in death
- Is life-threatening for the subject, life threatening events are defined as:
  - Circulatory/cardiac insufficiency requiring catecholamines/positive inotropes
  - Respiratory failure requiring intubation/ventilation

- Other clinical situation requiring immediate intervention, e.g. gastro-intestinal bleeding or perforation requiring surgery, cerebral abcessbleeding requiring immediate neurosurgical intervention.
- Requires hospitalisation or prolongation of existing inpatients' hospitalisation
- Results in persistent or significant disability or incapacity
- Any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator

We will only register SAE's that have a possible or definite relationship with the investigational medical devices from informed consent up till 30 days after the last study lock was given to the patient (Serious Adverse Device Events = SADEs). Registration of all SAE's will lead to too many registrations in this patient group. These SADE's must be registered in HiX by the research nurse/researcher and on SADE report forms in Castor EDC by local data-management. Within 24 hours these SADE forms must be sent to the safety desk of the sponsor.

The causality assessment is made using the following:

- Not related: There is no evidence to suggest a causal relationship.
- Possible: There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time frame after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
- Definitely: There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

The sponsor will report the SADEs to the accredited METC that approved the protocol through the web portal ToetsingOnline (TOL). During this study we are not obliged to report SADEs to the Inspectie Gezondheidszorg en Jeugd (IGZ).

- SADEs that result in death or are life threatening and where a possible/definite causal relationship with the investigational product is suspected, need to be reported through ToetsingOnline within 7 days of first knowledge, followed by a maximum period of 8 days to complete the initial preliminary report.
- All other SADEs, where a possible/definite causal relationship with the investigational product is suspected, will be reported within a maximum period of 15 days after the sponsor has first knowledge of the serious adverse events.

SADEs will be evaluated with the SADE evaluation form. It will be determined if the SADE was anticipated (ASADE) or unanticipated (USADE).

8.3 Follow-up of Serious Adverse Device Events

SADEs need to be reported till 30 days after the last lock was given to the patient, as defined in the protocol. All SADEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

8.4 Data Safety Monitoring Board (DSMB)

A DSMB will be established to safeguard the interests of trial participants, assess the safety and efficacy of the interventions during the trial, and monitor the overall conduct of the clinical trial. Details can be found in the "*DSMB Charter of the CATERPILLAR-study"*. The interim-analysis is described in chapter 9.3.

The DSMB should receive and review the progress and accruing data of this trial and provide advice on the conduct of the trial to the Trial Steering Committee (TSC). The DSMB should inform the Chair of the steering committee if, in their view: The results are likely to convince a broad range of clinicians, including those supporting the trial and the general clinical community, that one trial arm is clearly indicated or contraindicated, and there was a reasonable expectation that this new evidence would materially influence patient management; or It becomes evident that no clear outcome would be obtained.

DSMB meetings:

1. Prior to the study start a meeting will be scheduled, to discuss the protocol, trial, analysis plan, future meetings, and to have the opportunity to clarify any aspects with the principal investigators.
2. A closed meeting will be scheduled after the inclusion of 231 patients, approximately 14.5 months after the study start. The efficacy and safety data (interim analysis) will be presented. Accumulating information relating to the recruitment and data quality, toxicity details based on pooled data, and total numbers for the primary outcome measure and other outcome measures may be presented, at the discretion of the DSMB.
3. At the end of the study a meeting will be scheduled to allow the DSMB to discuss the final data with the principal investigator.

The members of the DMC for this trial will be:

1. Dr. Marieke Witvliet, Pediatric Surgeon, Wilhelmina Children's Hospital, Utrecht, the Netherlands.
2. Dr. Bart Rijnders, Infectious Diseases, Erasmus Medical Center, Rotterdam, the Netherlands.
3. Prof. Dr. Hein Putter, Medical Statistician, Leids University Medical Center, Leiden, the Nethterlands.

The chair will be: Dr. Marieke Witvliet

The advice(s) of the DSMB will be sent to the principal investigator (Prof. Dr. M.H.W.A. Wijnen) of the study. Should the principal investigator decide not to fully implement the advice of the DSMB, the principal investigator will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

9. STATISTICAL ANALYSIS

The primary data analyses will be performed with the intention to treat principle (i.e. inclusion of all patients that were randomized). Additionally, a per-protocol analysis will be performed excluding patients who were not included within one week after CVC insertion, patients who never received the intervention and patients who missed three or more of the minimal amount (once every three weeks) of locks during the follow-up period. Categorical data will be presented as contingency tables (frequencies and percentages). For continuous data summary statistics of mean, standard deviation, median, minimum, and maximum will be presented. Differences between treatment groups with respect to baseline characteristics will be analyzed by using a Chi-square (or Fisher Exact in the presence of small numbers), and t-test for categorical or continuous variables respectively. In case of violation of the normality assumption a non-parametric test such as the Wilcoxon rank test will be applied.

9.1 Primary study parameter

For the primary outcome, the percentages and incidence rates (IR) of first CLABSIs per 1,000 CVAD-days will be reported for both study groups and compared by computing an IRR. The exact confidence limits for the IRRs will be based on the polynomial algorithm for person time data (85, 86). The nominal alpha level for the primary outcome in the final analysis will be equal to 0.045 due to the interim analysis (66-71).

9.2 Secondary study parameters

The cumulative incidence of CLABSI from CVAD insertion will be estimated by using a competing risk model (87) with CVAD removal due to non-CLABSI related reasons or death as competing events. To assess the difference between the cumulative incidence for the intervention (TCHL) and control (HL) group, the Gray's test will be used. (88)

To estimate the effect of risk factors on the occurrence of CLABSI, a Cox specific proportional hazard regression model from CVAD insertion will be estimated. Well known time fixed risk factors for a CLABSI to be incorporated into the model are diagnosis (hematological disease versus other diagnoses), CVAD type (TIVAP versus tunneled external CVADs) . Furthermore, TPN administration will be used in the model as time-dependent covariate). (87)

A landmark analysis at 28 days after CVAD insertion will be performed. The same risk factors as discussed above will be incorporated in the Cox specific hazard regression model with additional covariate number of lock days. The landmark point of 28 days was chosen based on clinical reasons, the first lock should have been given within the first four weeks after CVAD insertion.(89)

For the secondary outcomes, the percentages and IRs per 1,000 CVAD-days will be reported and compared by computing IRRs.

9.3 Interim analysis

After complete follow-up of the first 231 patients an interim analysis will be performed by the trial statistician. After the interim analysis is performed, the results will be presented at the second DSMB meeting, see chapter 8.4. The stopping rule is based on testing the one-sided test at $\alpha = 0.025$ for H_0: 'experimental incidence ≥ control incidence' against H_1: 'experimental incidence < control incidence'. The test is one-sided because there is no need to prove superiority of the control treatment in case it is better than the experimental. The stopping rule allows stopping for acceptance of the alternative hypothesis (superiority) as well as stopping for acceptance of the null hypothesis (futility). The stopping boundaries are based on choices of the α- and β-spending functions. The α-spending function determines how eager or reluctant one is to stop the trial for superiority. The β-spending function determines how eager or reluctant one is to stop the trial because the chance has become small that superiority can be concluded if the trial is continued. As α-spending function we have chosen the Jennison and Turnbull power family function with $\rho = 2.35$. This choice implies that the trial is stopped after 231 patients if the one-sided P-value is smaller than 0.005 (or 0.01 two-sided) in favor of the experimental treatment. As β-spending function we have chosen the Jennison and Turnbull power family function with $\rho = 3.2$. This choice implies stopping the trial after 231 patients if the one-sided P-value is ≥ 0.5, i.e. if the estimated treatment effect at that time is in favor of the control treatment.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, and October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO), General Data Protection Regulation (GDPR), Medical Treatment Contracts Act (WGBO), Medical Devices Act (Wmh), and Medical Devices Decree.

10.2 Recruitment and consent

If a patient will receive a tunneled CVAD, the surgeon/researcher will inform the patient and parents/legal guardian about the CVAD insertion procedure and the CATERPILLAR-study. After the verbal information has been given, the information will also be given to the patient and parents/legal guardian in writing. The patient and parents/legal guardian can determine if they want to participate in the study until the CVAD is inserted for <1 week. However, if this is not possible due to clinical circumstances, informed consent can be given within 4 weeks after CVAD insertion. The time to consideration depends on the date of insertion and the hospital admission duration after the CVAD insertion. The time to consideration is at least one day. If the patient and parents/legal guardian agree to participate in the study, the informed consent form will be signed. Additionally, the patient and parents/legal guardian will be asked if they want the researcher to inform all treating physicians/pharmacist about the trial participation, and if, after the completion of the trial, the researcher can ask the patient and parents/legal guardian if they are interested in participating in follow-up studies.

10.3 Benefits and risks assessment, group relatedness

As already described in the introduction, hypothetically, the TCHL will reduce the CLABSI rate. Therefore, the TCHL may reduce the administration of antibiotics, result in lower rates of CVAD removal, fewer days of hospital/ICU admission, and a reduced mortality rate due to CLABSI. Additionally, patients can benefit directly from reduced and more appropriate antibiotic use, without the risk of antibiotic resistance development. (12-41, 43-50)

The expected side effects are temporarily, caused by a spill-over of citrate, described if the TCHL is instilled too fast, and if the TCHL is accidentally flushed instead of aspirated: perioral dysesthesia, discomfort of neck and chest, dygeusia, nausea and vomiting. (23, 34, 35, 37, 39, 40, 45, 46, 48, 50) Additionally, hypersensitivity reactions, and heparin induced thrombocytopenia are possible side effects, but were not observed in the studies evaluated. A more frequent dispense of thrombolytics has been associated with lock solutions containing taurolidine and citrate in haemodialysis patients. (18, 20-22, 25, 26) However, this was only observed without the addition of heparin. (45-50) In this study, for the prevention of the above stated possible side-effects, the lock volumina are adjusted to the lumen of the CVAD that is inserted, the locks will be aspirated before instillation of a new lock, the locks will be instilled slowly (<1 ml per second), and an ultrasound of the insertion veins to detect CVTs will be performed if CVT related symptoms are observed.

In conclusion, we think the possible positive effects of the TCHL outweigh the remaining minimal and rare side effects of the TCHL.

10.4 Compensation for injury

The sponsor has a liability insurance which is in accordance with article 7 of the WMO. Insurance information:

- Insurance company: Aon Risk Solutions
- Type of Insurance: Liability Insurance (including medical malpractice liability).
- Policy no: V0100112728
- Insured: Prinses Maxima Centrum voor Kinderoncologie
- Sum insured: EUR 5,000,000 each and every claim and EUR 15,000,000 in the aggregate.
- Deductible: EUR 25,000 each and every claim
- Insurance period: May 18, 2019 till May 18, 2020
- Conditions: In conformity with the AW Healthcare package wording, including general liability, pollution (sudden & accident) and employer's liability (Dutch law). Further to be agreed and amended to Dutch law.
- Territorial limits: Worldwide, excluding USA/Canada
- Leading insurer: 100% Allied World Assurance Company (Europe) Ltd.

The sponsor also has an insurance for the study subjects which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

Insurance information:

- Insurance company: CNA Insurance Company, Ltd
- Type of Insurance: Subject insurance
- Policy no: 10211864
- Insured: Prinses Maxima Centrum voor Kinderoncologie
- Sum insured: EUR 650,000 per subject, EUR 5,000,000 per research project, EUR 7,500,000 each year for all research projects together.
- Insurance period: October 1, 2019 till October 1, 2020, with silent prolongation.
- Territorial limits: The Netherlands

10.5 Incentives

No incentives/compensations are applicable.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

The handling of the personal data will comply with the General Data Protection Regulation (GDPR). All data will be handled confidentially and pseudonymised. The database system that we will use is Castor EDC (www.castoredc.com), a user friendly, fully featured, affordable and secure system. Castor has been audited on Good Clinical Practice compliance by Profess Medical Consultancy and has obtained a Good Clinical Practice compliance certificate. The database will have limited excess; an account will be given to the members of the local study team and to a designated monitor. A central subject identification code list in the Princess Máxima Center for Pediatric Oncology will be used to link the data to the subject. The subject identification list will only be available for the local study team. The database and the subject identification list will be kept separately. Data will be stored in the Princess Máxima Center for Pediatric Oncology for a minimum of 15 years.

11.2 Monitoring and Quality Assurance

The monitor organisation: Julius Center (http://portal.juliuscentrum.nl/nl-nl/home.aspx)
Independence of the organisation: The Julius Center is an organisation of the University Medical Center Utrecht which supports research. The Julius Center is not depended on the outcomes of this trial.

Risk classification
Negligible risk

Monitoring frequention
An independent monitor will make one prior to start visit, one site visit in the Princess Máxima Center each four months, and one close-out visit.

Monitoring plan
Study documents and agreements:

- Confirming that the research file is present and complete: Trial Master File and Investigator File.
- Confirming that the study staff is completely instructed on the study procedures, and that back-up agreements are made with other colleagues.

Patient inclusion rate, consent, compliance and Source Document Verification (SDV):

- Checking the inclusion rate and drop-out percentage.
- Checking the informed consent papers: sample of 10%
- Checking the in- and exclusion criteria: sample of first three subjects, afterwards 1-10%
- Checking the protocol compliance: sample of the first three subjects, afterwards 1-10%
- Source Document Verification (SDV): sample of 1-10%; will be performed for a predefined list list of variables which have a clear relationship to the safety and validity of the research (including the primary end-point).

Patient safety

- Verification of Serious Adverse Event (SAE) reporting: sample of 1-10% of the subjects.

Investigational product
- Verification of the patient instructions that are given.

Study procedures
- Verification if the study procedure instructions are accessible.

Laboratory and pharmacy
- Verification if the laboratory is GLP certified
- Verification if the pharmacy is GMP certified

Attention points
- Qualifications of the monitor
- Feedback and follow-up of the observations of the monitor
  - Term of monitor report availability
  - Actions regarding the points of improvement in the monitoring report within the Princes Máxima Center.
- Storage of study files
  - Use of an adequate Clinical Data Management System (CDMS).
  - Correct storage of raw data, corrected data, and back-ups.
  - Presence of an audit trail.

Monitoring reports and storage period
A monitoring report will be written of every monitoring visit. The head of the department of the researcher is responsible for archiving the reports for a minimum of 15 years after the end of the study. The monitoring report and other study documents are available for the Board of Directors of the Princess Máxima Center for pediatric oncology and for the employees assigned by the Board of Directors.

11.3 Amendments
Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

11.4 Annual progress report
The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

11.5 Temporary halt and (prematurely) end of study report
The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit. The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

11.6 Public disclosure and publication policy

The results of this research will be disclosed unreservedly. All parties concerned must justify their actions in this regard. Patients and human subjects are entitled to public disclosure of the results of the trial on the basis of their participation in it (and the arguments that play a role therein).

Both positive and negative trial results will be disclosed. The results of research will be submitted for publication to open access peer-reviewed scientific journals. If the journals do not consider negative results for publication, the research will be disclosed through trial registers, websites or databases.

The basic principles of the Vancouver convention (Uniform requirements for manuscripts submitted to biomedical journals. JAMA 277:927-934,1997) and the editors' statements of a number of authoritative biomedical scientific journals (Davidoff F et al., Sponsorship, authorship and accountability, NEJM 345:825-826, 2001) will be followed.

The sponsor is entitled to examine the manuscript prior to publication and to make comments on it. The sponsor may delay publication for up to three months after analysing the research results.

Disputes will be dealt with by continuing the debate in the form of letters sent to the scientific journal.

None of the parties concerned has a right of veto. The parties concerned must attempt to resolve disputes by negotiation. Should one of the parties feel that it has been disadvantaged, or should any other problem relating to publication arise, the parties can contact the METC for mediation.

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

Chapter 12.1 is not applicable as the investigational medical device is registered and used within the registered indication.

12.2 Synthesis

Hypothetically, the TCHL will reduce the CLABSI rate compared to the HL. Therefore, the TCHL may reduce the administration of antibiotics, result in lower rates of CVAD removal, fewer days of hospital/ICU admission, and a reduced mortality rate due to CLABSI compared to the HL. Additionally, patients will benefit directly from reduced and more appropriate antibiotic use, without the risk of antibiotic resistance development. (12-20, 45-48, 50, 59, 62)

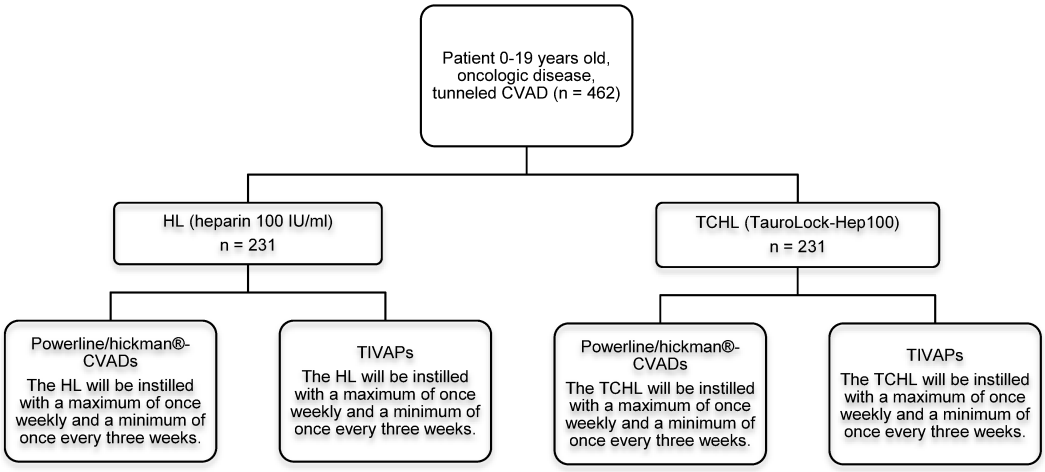
The expected side effects are temporarily, caused by a spill-over of citrate, described if the TCHL is instilled to fast, and if the TCHL is accidentally flushed instead of aspirated: perioral dysesthesia, discomfort of neck and chest, dygeusia, nausea and vomiting. (23, 34, 35, 37, 39, 40, 45, 46, 48, 50) Additionally, hypersensitivity reactions, and heparin induced thrombocytopenia are possible side-effects, but only in one patient an anaphylactic-like reaction was observed. (34) Liver-injury is associated with high-concentrations of systemic taurolidine in mouse-models. The TCHL contains low-dose taurolidine, which is not associated with liver-injury. (63) A more frequent dispense of thrombolytics has been associated with lock solutions containing taurolidine and citrate in haemodialysis patients. (18, 20-22, 25, 26) However, this was only observed without the addition of heparin. (45-50) For the prevention of the above stated possible side-effects the lock volumina are adjusted to the lumen of the CVAD that is inserted, the locks will be aspirated before instillation of a new lock, the locks will be instilled slowly (<1 ml per second), heparin is added to the solution for the prevention of the more frequent dispense of thrombolytics, and an ultrasound of the insertion veins to detect CVTs will be performed if CVT related symptoms are observed during the study. The locks will be instilled with a maximum of once weekly and a minimum of once every three weeks. After every study-lock instillation, the patients will be asked to answer a questionnaire about the experience of possible side effects.

In conclusion, we think the possible positive effects of the TCHL outweigh the remaining minimal and rare side effects of the TCHL. We hope to prove that the TCHL will reduce the CLABSI rate, CVAD-removal rate, dispense of antibiotics, days of hospital/intensive care unit admission, and mortality rate due to CLABSI.

13. APPENDICES

13.1 Appendix 1: Flow-chart lock solutions

In between the study locks, the patients will receive heparin 100 IU/ml locks.



Endpoints of the study, whatever endpoint will come first:

- First tunneled CLABSI
- Removal of the CVAD
- Second CVAD insertion (excl. stem cell apheresis CVADs)
- Death of the patient
- Study period of 90 days

13.2 Appendix 2: TauroLock-Hep100 Documents

ENGLISH **Instructions For Use** 43703GB/14/17

TauroLock™ HEP 100

Catalogue # TP-03

A. Description and Specifications
TauroLock™-HEP100 contains anticoagulants and antimicrobial substances. It is to be used with a port or a catheter-based vascular access device. It is to be instilled in the device lumens between treatments in order to make the internal flow passages resistant to clot formation and hostile to bacterial and fungal growth. The solution must be withdrawn prior to initiating the next treatment. Active ingredients in TauroLock™-HEP100 are (cyclo)-taurolidine, citrate (4%) and heparin (mucosa, 100 IU/mL). Other components include water for injection. The pH is adjusted with citrate and/or sodium hydroxide. The product is sterile filter processed and supplied as a clear, sterile, non-pyrogenic solution.
Note:
For complete details of catheter-based vascular access products, consult the manufacturer's instructions or clinician's manual.

B. Indications
TauroLock™-HEP100 is indicated for those patients who use a port or a silicone or polyurethane catheter-based device as vascular access. TauroLock™-HEP100 is intended to be used as a catheter lock solution. It is to be instilled into the device at the termination of a treatment and withdrawn prior to initiating subsequent treatments (see F4).

C. Contraindications
TauroLock™-HEP100 is contraindicated for patients with a known allergy to (cyclo)-taurolidine, citrate or heparin (mucosa) or when a patient is currently taking medication with known adverse interaction to citrate, heparin or (cyclo)-taurolidine. TauroLock™-HEP100 is also contraindicated for patients with heparin-induced thrombocytopenia or increased bleeding risk.

D. Cautions
1. As a consumable TauroLock™-HEP100 is for single use only. Reuse creates a potential contamination risk for the patient.
2. TauroLock™-HEP100 is not for systemic injection. TauroLock™-HEP100 must be used as a catheter lock solution as described in the access device's instruction for use. Failure to adhere to these instructions may result in inadvertent systemic injection of the solution. Once instilled into the catheter the solution must not be used again after aspiration.
3. The ampoule is for single dose only due to potential risk of contamination.
4. Some patient populations using TauroLock™-HEP100 antimicrobial lock solution may experience a higher frequency of blood clots in the catheter lumen. In the event that access device patency is compromised, follow institutional protocol for restoring flow.
5. The specific fill volume of the access device has to be strictly respected with infants and children less than two years of age due to citrate as an active ingredient.
6. In access devices which were blocked regularly with non-antimicrobial lock solutions (e.g. with heparin, low concentrated citrate or saline) prior to application of TauroLock™-HEP100, viable organisms and endotoxins may be released from the biofilm. The lock solution must be aspirated before the next treatment to prevent very rare anaphylactic reactions which are not attributable to the active ingredients.
7. The concentration of the antimicrobial compound is near to saturation. If not stored or transported according to the instructions under section H, precipitation can occur in the product. Do not use such a precipitated product.

E. Adverse Effects
To date, there are no known adverse effects in humans due to the active ingredient concentrations in TauroLock™-HEP100 when used as directed. There are no known risks associated with concomitant systemic antibiotic therapy or exposure to magnetic fields. TauroLock™-HEP100 may cause mild hypocalcaemic symptoms if instillation is not done slowly as directed.

F. Instillation of TauroLock™-HEP100
Follow the manufacturer's instructions that accompany the particular vascular access product utilized. Specific catheter lock volumes are associated with each device.
1. Flush the device with 10 mL of saline.
2. Withdraw TauroLock™-HEP100 from the container using an appropriate syringe.
3. Instill TauroLock™-HEP100 slowly (not more than 1 mL per second, infants and children less than two years of age not more than 1 mL per 5 seconds) into the access device in a quantity sufficient to fill the lumen completely. **Consult the manufacturer's instructions for the specific fill volume or specify fill volume during implantation. The volume has to be strictly respected.** TauroLock™-HEP100 will remain inside the access device until the next treatment (for a maximum of 30 days).
4. Prior to the next treatment, TauroLock™-HEP100 must be aspirated and discarded according to the institution's waste policy. Prior to initiation of the next treatment, TauroLock™-HEP100 must be withdrawn from the access device and discarded according to the institution's waste policy.
5. Flush the device with 10 mL of saline.

G. Pregnancy and Breastfeeding
No data are available for pregnant and breastfeeding women. For safety reasons TauroLock™-HEP100 should not be used during pregnancy and breastfeeding.

H. Storage and shipment
TauroLock™-HEP100 must be stored at a temperature of 15 to 30°C and must not be shipped at freezing temperature. Do not freeze.

I. Packaging configuration
The following packaging configurations are available for TauroLock™-HEP100: 10 x 3 mL TauroLock™-HEP100 ampoules.

State: 07. December 2015

TauroPharm GmbH · August-Bebel-Straße 51 · D-97297 Waldbüttelbrunn · Germany
Tel: +49 931 304 299 0 · Fax: +49 931 304 299 29

STERILE A Sterile, aseptic fill.
Read instruction for use.
Single use. The ampoule is a single dose.
Do not use when package is damaged.
CE 0123 CE acc. MDD 93/42/EEC, notified body: TÜV SÜD PRODUCT SERVICE GmbH.

TauroPharm GmbH
August-Bebel-Str. 51
D-97297 Waldbüttelbrunn
Germany

Tel + 49 (931) 304 299 0
Fax + 49 (931) 304 299 29

DECLARATION OF CONFORMITY

MANUFACTURER:	TauroPharm GmbH August-Bebel-Str. 51 D-97297 Waldbüttelbrunn, Germany
PRODUCT:	TauroLock™-HEP100 (3 ml ampoule)
CLASSIFICATION:	III
CONFORMITY ASSESSMENT ROUTE:	Annex II

We herewith declare that the above mentioned products meet the provisions of the Council Directive 93/42/EEC for medical devices. All supporting documentation is retained under the premise of the manufacturer.

STANDARDS APPLIED:	MDD 93/42 EEC
NOTIFIED BODY:	TÜV SÜD Product Service GmbH Ridlerstrasse 65 D-80339 Munich, Germany Reg. No. 0123
EC CERTIFICATE:	G1 17 05 51963 014 G7 17 06 51963 020
START OF CE-MARKING:	This declaration applies to all CE-marked devices manufactured from the date of issuance until it is either superseded by another declaration or withdrawn.
ISSUED BY:	This Declaration of Conformity is issued by TauroPharm GmbH, which is exclusively responsible for the declared compliance.
PLACE OF ISSUE:	TauroPharm GmbH, D-97297 Waldbüttelbrunn, Germany
SIGNATURE:	..... (Dr. Christian Weis, Managing Director)
DATE:	31. July 2017

TauroPharm GmbH
August-Bebel-Straße 51
97297 Waldbüttelbrunn
Tel. 0049931/304299-0
GERMANY

ZERTIFIKAT ◆ CERTIFICATE ◆ 認証書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

Product Service

EC Certificate

EC Design-Examination Certificate

Directive 93/42/EEC on Medical Devices (MDD), Annex II (4)
(Devices in Class III)

No. G7 17 06 51963 020

Manufacturer:

TauroPharm GmbH
August-Bebel-Str. 51
97297 Waldbüttelbrunn
GERMANY

Product:

Irrigation Solutions
Non antibiotic based antimicrobial catheter lock solution

The Certification Body of TÜV SÜD Product Service GmbH declares that a design examination has been carried out on the respective devices in accordance with MDD Annex II (4). The design of the devices conforms to the requirements of this Directive. For marketing of these devices an additional Annex II certificate is mandatory. See also notes overleaf.

Report no.: 713104720

Valid from: 2017-07-31
Valid until: 2022-07-30

Date, 2017-07-28

Stefan Preiß

TÜV SÜD Product Service GmbH is Notified Body with identification no. 0123

Page 1 of 2

TÜV SÜD Product Service GmbH · Zertifizierstelle · Ridlerstraße 65 · 80339 München · Germany

TÜV®

A1 / 04.11

ZERTIFIKAT ◆ CERTIFICATE ◆ 認証証書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

Product Service

EC Certificate
EC Design-Examination Certificate

Directive 93/42/EEC on Medical Devices (MDD), Annex II (4)
(Devices in Class III)

No. G7 17 06 51963 020

Model(s):

Taurolock Solutions
- Taurolock Hep TP-02
- Taurolock Hep TP-03

Parameters:

Taurolock with Heparin 500:	TP-02 3ml, 5ml Ampoule, 10 ml Vial
Taurolock with Heparin 100:	TP-03 3ml, 5ml Ampoule, 10 ml Vial

Facility(ies):

TauroPharm GmbH
August-Bebel-Str. 51, 97297 Waldbüttelbrunn, GERMANY

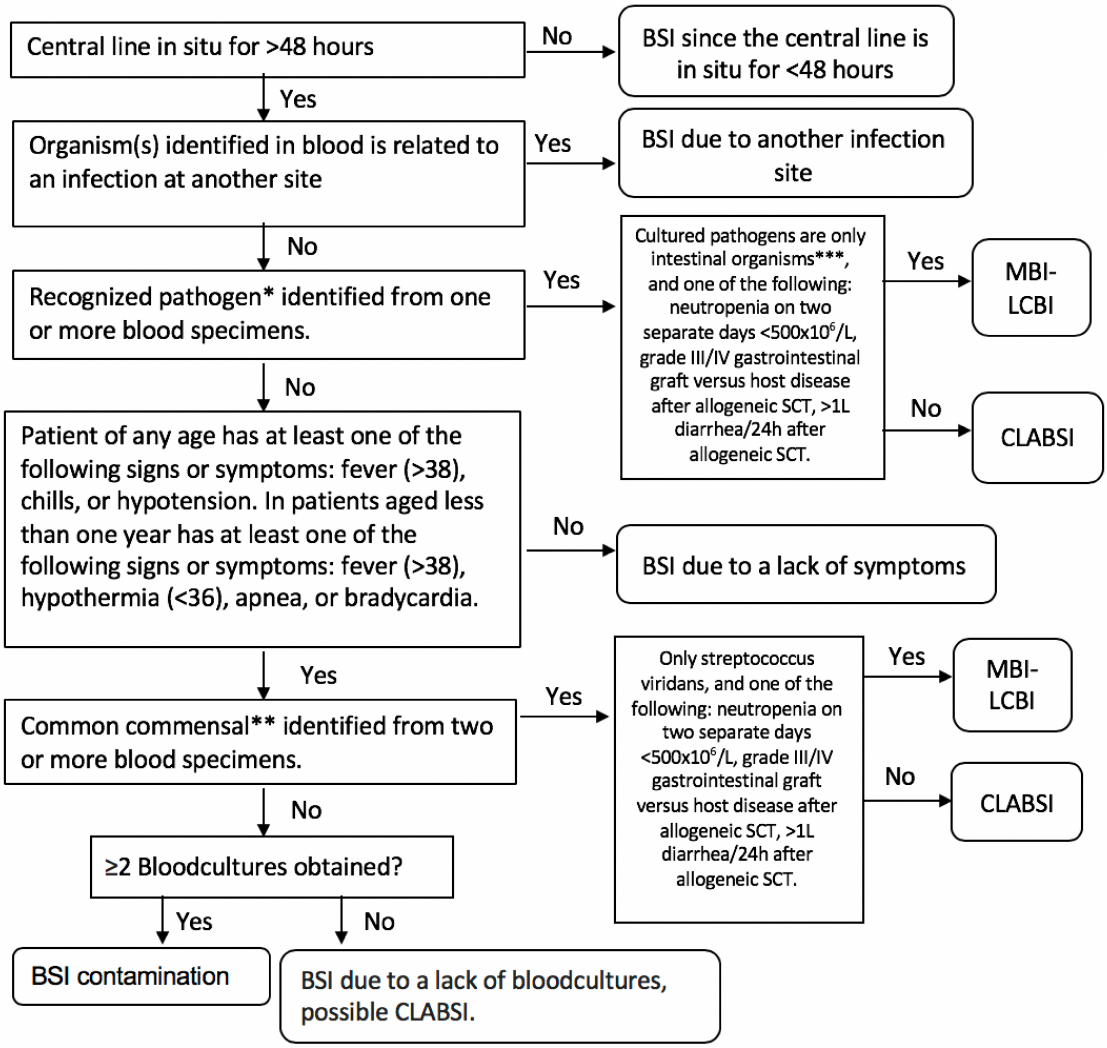
Page 2 of 2

A1 / 04.11

TÜV SÜD Product Service GmbH · Zertifizierstelle · Ridlerstraße 65 · 80339 München · Germany

TÜV®

13.3 Appendix 3: Flow-chart suspicion of a CLABSI



CVAD = Central Venous Access Device, BSI = Bloodstream Infection, MBI-LCBI = Mucosal Barrier Injury – Laboratory Confirmed Bloodstream Infection, CLABSI = Central Line Associated Bloodstream Infection, SCT = Stem Cell Transplantation.

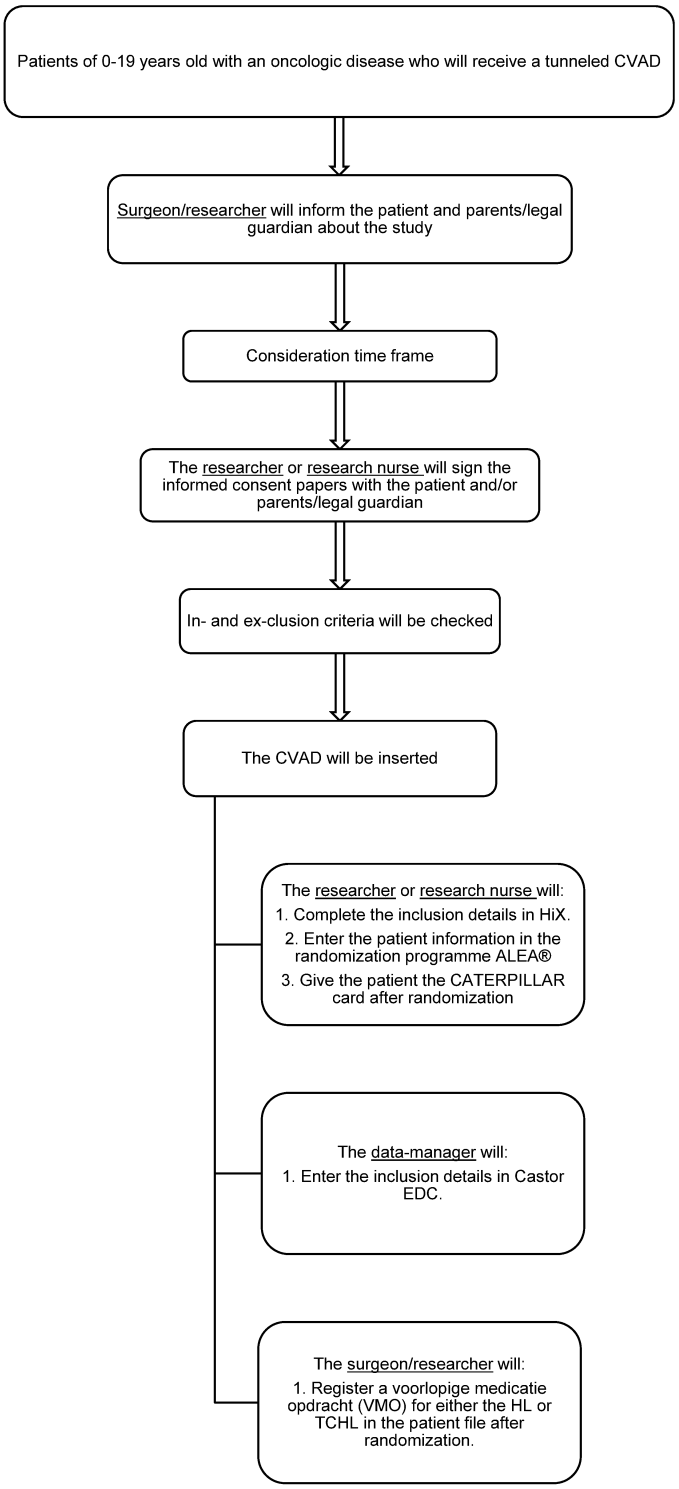
* Recognized pathogens are pathogens that are not included on the NHSN common commensal list (e.g. *S. Aureus*): https://nhsn.cdc.gov/nhsntraining/courses/2016/C18/page3299.html. The following micro-organisms are not included in the common commensal list but are not recognized pathogens: *Campylobacter, C. difficile, Enterpopathogenic E. coli, Listeria spp., Salmonella spp.*, en *Yersinia spp.*

** Common commensals are micro-organisms that are included on the NHSN common commensal list (e.g. *Coagulase-negative staphylococci, Viridians group streptococci, Bacillus spp., Diphtheroids, Aerococcus spp. Micrococcus spp.)*: https://nhsn.cdc.gov/nhsntraining/courses/2016/C18/page3299.html.

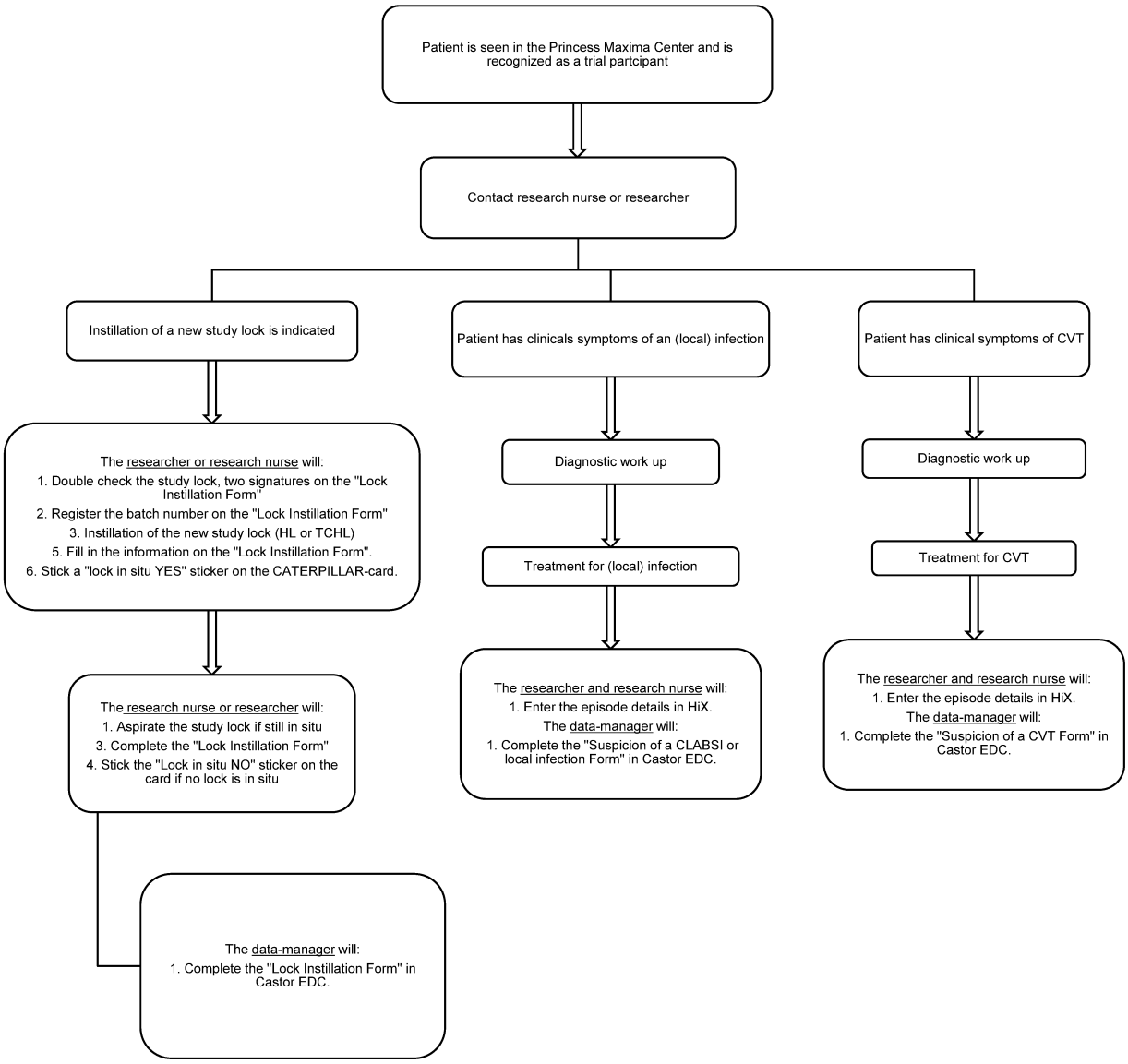
*** Micro-organisms registered as MBI Organisms on the NHSN common commensal list (e.g. *Escherichia coli, Enterobacteriaceae, and Enterococci)*: https://nhsn.cdc.gov/nhsntraining/courses/2016/C18/page3299.html.

**** Viridans streptococci: e.g. *S. mitis, S. oralis, S. salivarius, S. thermophilus, S. vestibularis, S. anginosus, S. sanguinis, S. parasanguinis, S. gordonii, S. mutans, en S. sobrinus.*

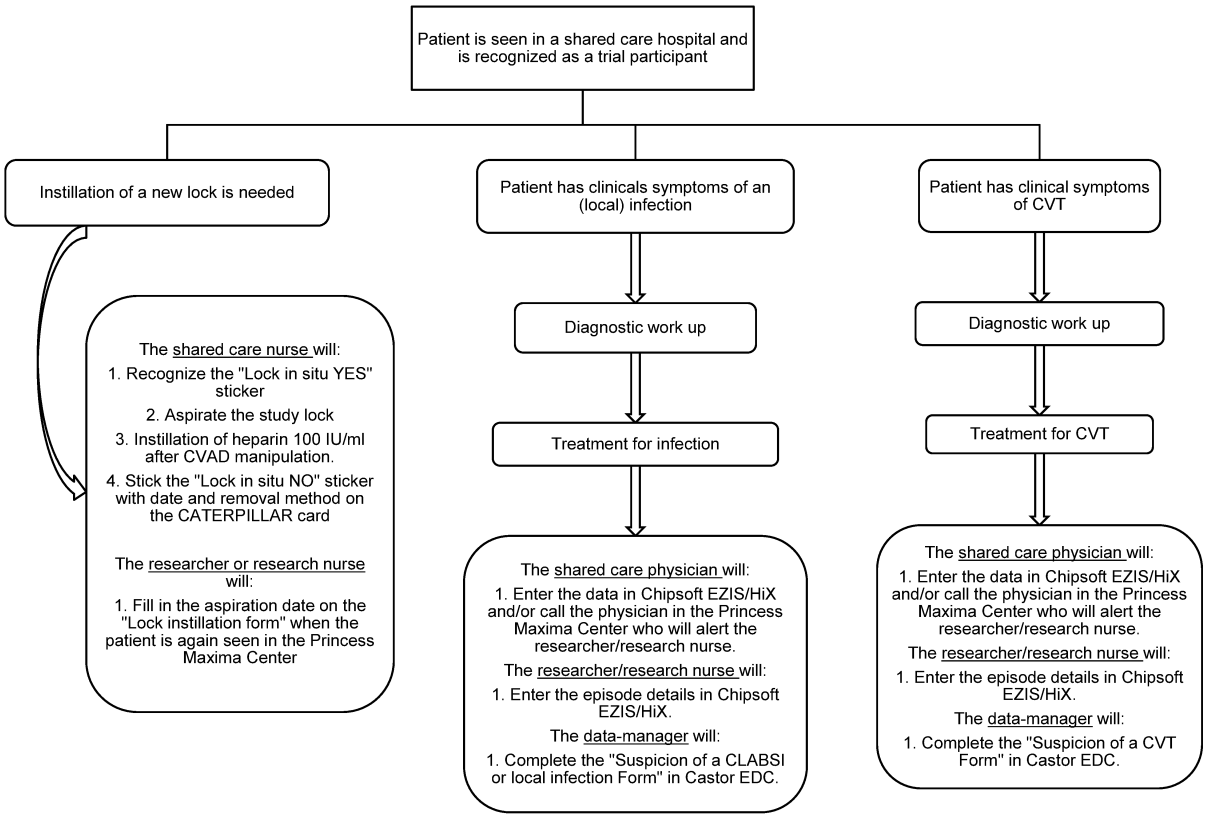
13.4 Appendix 4: Flow-chart study procedure



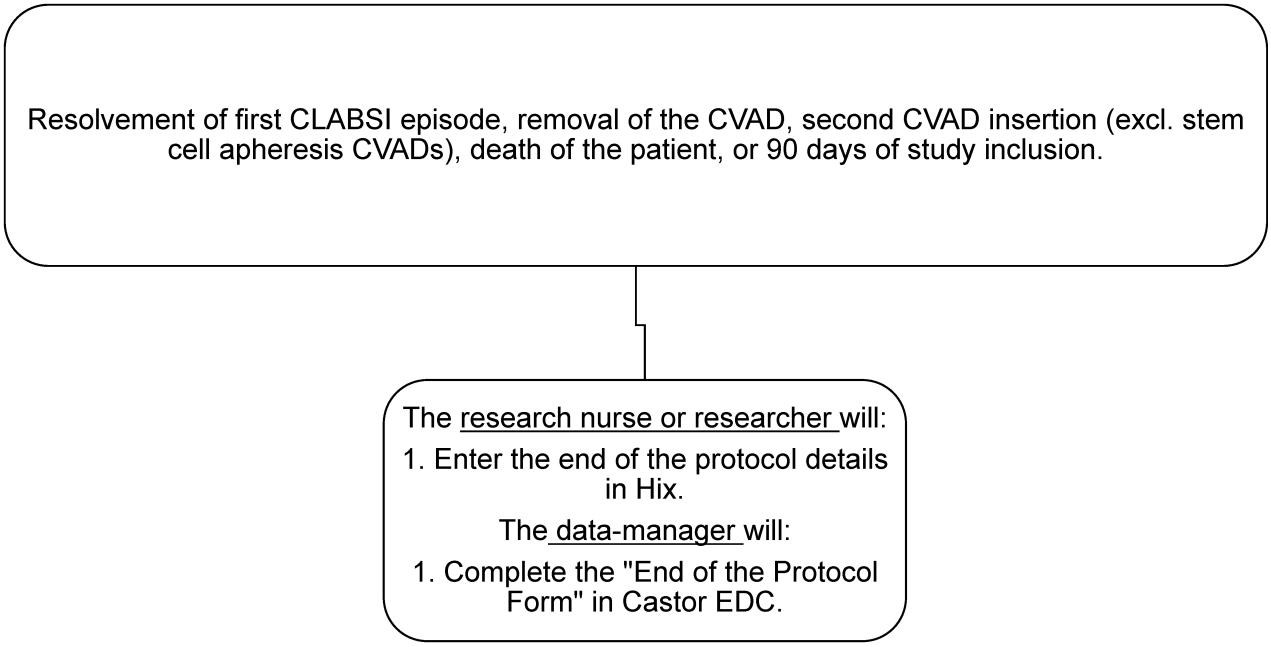
13.5 Appendix 5: Flow-chart study procedure Princess Máxima Center



13.6 Appendix 6: Flow-chart study procedure Shared Care Hospitals



13.7 Appendix 7: End of the Protocol flow-chart



14. REFERENCES

1. van den Bosch CH, van der Bruggen JT, Frakking FNJ, Terwisscha van Scheltinga CEJ, van de Ven CP, van Grotel M, et al. Incidence, severity and outcome of central line related complications in pediatric oncology patients; A single center study. J Pediatr Surg. 2019;54(9):1894-900.
2. Schoot RA, van Ommen CH, Stijnen T, Tissing WJ, Michiels E, Abbink FC, et al. Prevention of central venous catheter-associated bloodstream infections in paediatric oncology patients using 70% ethanol locks: A randomised controlled multi-centre trial. Eur J Cancer. 2015;51(14):2031-8.
3. O'Grady NP, Alexander M, Burns LA, Dellinger EP, Garland J, Heard SO, et al. Guidelines for the prevention of intravascular catheter-related infections. Am J Infect Control. 2011;39(4 Suppl 1):S1-34.
4. Schoot RA vDE, van Ommen CH, van de Wetering MD. Antibiotic and other lock treatments for tunnelled central venous catheter-related infections in children with cancer. Cochrane Database of Systematic Reviews. 2013(6).
5. Cesaro S, Cavaliere M, Pegoraro A, Gamba P, Zadra N, Tridello G. A comprehensive approach to the prevention of central venous catheter complications: results of 10-year prospective surveillance in pediatric hematology-oncology patients. Ann Hematol. 2016;95(5):817-25.
6. Fratino G, Molinari AC, Parodi S, Longo S, Saracco P, Castagnola E, et al. Central venous catheter-related complications in children with oncological/hematological diseases: an observational study of 418 devices. Ann Oncol. 2005;16(4):648-54.
7. Pektas A, Kara A, Gurgey A. Cohort Study: Central Venous Catheter-Related Complications in Children with Hematologic Diseases at a Single Center. Turk J Haematol. 2015;32(2):144-51.
8. Pinon M, Bezzio S, Tovo PA, Fagioli F, Farinasso L, Calabrese R, et al. A prospective 7-year survey on central venous catheter-related complications at a single pediatric hospital. Eur J Pediatr. 2009;168(12):1505-12.
9. Pittiruti M, Bertoglio S, Scoppettuolo G, Biffi R, Lamperti M, Dal Molin A, et al. Evidence-based criteria for the choice and the clinical use of the most appropriate lock solutions for central venous catheters (excluding dialysis catheters): a GAVeCeLT consensus. J Vasc Access. 2016;17(6):453-64.
10. Norris LB, Kablaoui F, Brilhart MK, Bookstaver PB. Systematic review of antimicrobial lock therapy for prevention of central-line-associated bloodstream infections in adult and pediatric cancer patients. Int J Antimicrob Agents. 2017;50(3):308-17.
11. Vassallo M, Dunais B, Roger PM. Antimicrobial lock therapy in central-line associated bloodstream infections: a systematic review. Infection. 2015;43(4):389-98.
12. Betjes MG, van Agteren M. Prevention of dialysis catheter-related sepsis with a citrate-taurolidine-containing lock solution. Nephrol Dial Transplant. 2004;19(6):1546-51.
13. Kavosi Z, Sarikhani Khorrami M, Keshavarz K, Jafari A, Hashemi Meshkini A, Safaei HR, et al. Is Taurolidine-citrate an effective and cost-effective hemodialysis catheter lock solution? A systematic review and cost- effectiveness analysis. Med J Islam Repub Iran. 2016;30:347.

14. Liu H, Liu H, Deng J, Chen L, Yuan L, Wu Y. Preventing catheter-related bacteremia with taurolidine-citrate catheter locks: a systematic review and meta-analysis. Blood Purif. 2014;37(3):179-87.
15. Fontsere N, Cardozo C, Donate J, Soriano A, Muros M, Pons M, et al. Tunneled catheters with taurolidine-citrate-heparin lock solution significantly improve the inflammatory profile of hemodialysis patients. Antimicrob Agents Chemother. 2014;58(7):4180-4.
16. Zwiech R, Adelt M, Chrul S. A Taurolidine-Citrate-Heparin Lock Solution Effectively Eradicates Pathogens From the Catheter Biofilm in Hemodialysis Patients. Am J Ther. 2016;23(2):e363-8.
17. Murray EC, Deighan C, Geddes C, Thomson PC. Taurolidine-citrate-heparin catheter lock solution reduces staphylococcal bacteraemia rates in haemodialysis patients. QJM. 2014;107(12):995-1000.
18. Allon M. Prophylaxis against dialysis catheter-related bacteremia with a novel antimicrobial lock solution. Clin Infect Dis. 2003;36(12):1539-44.
19. Taylor C, Cahill J, Gerrish M, Little J. A new haemodialysis catheter-locking agent reduces infections in haemodialysis patients. J Ren Care. 2008;34(3):116-20.
20. Solomon LR, Cheesbrough JS, Bhargava R, Mitsides N, Heap M, Green G, et al. Observational study of need for thrombolytic therapy and incidence of bacteremia using taurolidine-citrate-heparin, taurolidine-citrate and heparin catheter locks in patients treated with hemodialysis. Semin Dial. 2012;25(2):233-8.
21. Filiopoulos V, Hadjiyannakos D, Koutis I, Trompouki S, Micha T, Lazarou D, et al. Approaches to prolong the use of uncuffed hemodialysis catheters: results of a randomized trial. Am J Nephrol. 2011;33(3):260-8.
22. Geron R, Tanchilevski O, Kristal B. [Catheter lock solution--taurolock for prevention of catheter-related bacteremia in hemodialysis patients]. Harefuah. 2006;145(12):881-4, 943.
23. Reidenberg BE, Wanner C, Polsky B, Castanheira M, Shelip A, Stalleicken D, et al. Postmarketing experience with Neutrolin(R) (taurolidine, heparin, calcium citrate) catheter lock solution in hemodialysis patients. Eur J Clin Microbiol Infect Dis. 2018;37(4):661-3.
24. Sodemann K, Polaschegg HD, Feldmer B. Two years' experience with Dialock and CLS (a new antimicrobial lock solution). Blood Purif. 2001;19(2):251-4.
25. Solomon LR, Cheesbrough JS, Ebah L, Al-Sayed T, Heap M, Millband N, et al. A randomized double-blind controlled trial of taurolidine-citrate catheter locks for the prevention of bacteremia in patients treated with hemodialysis. Am J Kidney Dis. 2010;55(6):1060-8.
26. Winnicki W, Herkner H, Lorenz M, Handisurya A, Kikic Z, Bielesz B, et al. Taurolidine-based catheter lock regimen significantly reduces overall costs, infection, and dysfunction rates of tunneled hemodialysis catheters. Kidney Int. 2018;93(3):753-60.
27. Al-Amin AH, Sarveswaran J, Wood JM, Burke DA, Donnellan CF. Efficacy of taurolidine on the prevention of catheter-related bloodstream infections in patients on home parenteral nutrition. J Vasc Access. 2013;14(4):379-82.
28. Toure A, Lauverjat M, Peraldi C, Boncompain-Gerard M, Gelas P, Barnoud D, et al. Taurolidine lock solution in the secondary prevention of central venous catheter-associated bloodstream infection in home parenteral nutrition patients. Clin Nutr. 2012;31(4):567-70.

29. Bisseling TM, Willems MC, Versleijen MW, Hendriks JC, Vissers RK, Wanten GJ. Taurolidine lock is highly effective in preventing catheter-related bloodstream infections in patients on home parenteral nutrition: a heparin-controlled prospective trial. Clin Nutr. 2010;29(4):464-8.
30. Chu HP, Brind J, Tomar R, Hill S. Significant reduction in central venous catheter-related bloodstream infections in children on HPN after starting treatment with taurolidine line lock. J Pediatr Gastroenterol Nutr. 2012;55(4):403-7.
31. Lambe C, Poisson C, Talbotec C, Goulet O. Strategies to Reduce Catheter-Related Bloodstream Infections in Pediatric Patients Receiving Home Parenteral Nutrition: The Efficacy of Taurolidine-Citrate Prophylactic-Locking. JPEN J Parenter Enteral Nutr. 2018;42(6):1017-25.
32. Jurewitsch B, Jeejeebhoy KN. Taurolidine lock: the key to prevention of recurrent catheter-related bloodstream infections. Clin Nutr. 2005;24(3):462-5.
33. Klek S, Szczepanek K, Hermanowicz A, Galas A. Taurolidine lock in home parenteral nutrition in adults: results from an open-label randomized controlled clinical trial. JPEN J Parenter Enteral Nutr. 2015;39(3):331-5.
34. Olthof ED, Versleijen MW, Huisman-de Waal G, Feuth T, Kievit W, Wanten GJ. Taurolidine lock is superior to heparin lock in the prevention of catheter related bloodstream infections and occlusions. PLoS One. 2014;9(11):e111216.
35. Tribler S, Brandt CF, Petersen AH, Petersen JH, Fuglsang KA, Staun M, et al. Taurolidine-citrate-heparin lock reduces catheter-related bloodstream infections in intestinal failure patients dependent on home parenteral support: a randomized, placebo-controlled trial. Am J Clin Nutr. 2017;106(3):839-48.
36. Saunders J, Naghibi M, Leach Z, Parsons C, King A, Smith T, et al. Taurolidine locks significantly reduce the incidence of catheter-related blood stream infections in high-risk patients on home parenteral nutrition. Eur J Clin Nutr. 2015;69(2):282-4.
37. Taniguchi AE, J.; Davidson A.; Nightingale J.; Gabe S.M. . Effectiveness of TauroLock™ in preventing recurrent catheter-related bloodstream infections in patients on home parenteral nutrition. Proceedings of the Nutrition Society. 2009;68(OCE1):E58.
38. Hulshof EC, Hanff LM, Olieman J, de Vette S, Driessen GJ, Meeussen C, et al. Taurolidine in Pediatric Home Parenteral Nutrition Patients. Pediatr Infect Dis J. 2017;36(2):233-5.
39. Wouters Y, Roosenboom B, Causevic E, Kievit W, Groenewoud H, Wanten GJA. Clinical outcomes of home parenteral nutrition patients using taurolidine as catheter lock: A long-term cohort study. Clin Nutr. 2018.
40. Wouters Y, Theilla M, Singer P, Tribler S, Jeppesen PB, Pironi L, et al. Randomised clinical trial: 2% taurolidine versus 0.9% saline locking in patients on home parenteral nutrition. Aliment Pharmacol Ther. 2018.
41. Arnoriaga Rodriguez M, Perez de Ciriza Cordeu M, Camblor Alvarez M, Breton Lesmes I, Motilla de la Camara M, Velasco Gimeno C, et al. Clinical and economic impact of the taurolidine lock on home parenteral nutrition. Nutr Hosp. 2018;35(4):761-6.
42. Lyszkowska M, Kowalewski G, Szymczak M, Polnik D, Mikolajczyk A, Kalicinski P. Effects of prophylactic use of taurolidine-citrate lock on the number of catheter-related infections in children under 2 years of age undergoing surgery. J Hosp Infect. 2019;103(2):223-6.

43. Longo R, Llorens M, Goetz C, Platini C, Eid N, Sellies J, et al. Taurolidine/Citrate Lock Therapy for Primary Prevention of Catheter-Related Infections in Cancer Patients: Results of a Prospective, Randomized, Phase IV Trial (ATAPAC). Oncology. 2017;93(2):99-105.
44. Gudiol C, Arnan M, Aguilar-Guisado M, Royo-Cebrecos C, Sanchez-Ortega I, Montero I, et al. A Randomized, Double-Blind, Placebo-Controlled Trial (TAURCAT Study) of Citrate Lock Solution for Prevention of Endoluminal Central Venous Catheter Infection in Neutropenic Hematological Patients. Antimicrob Agents Chemother. 2020;64(2).
45. Dumichen MJ, Seeger K, Lode HN, Kuhl JS, Ebell W, Degenhardt P, et al. Randomized controlled trial of taurolidine citrate versus heparin as catheter lock solution in paediatric patients with haematological malignancies. J Hosp Infect. 2012;80(4):304-9.
46. Handrup MM, Moller JK, Schroder H. Central venous catheters and catheter locks in children with cancer: a prospective randomized trial of taurolidine versus heparin. Pediatr Blood Cancer. 2013;60(8):1292-8.
47. Ince E, Oguzkurt P, Temiz A, Ezer SS, Gezer HO, Yazici N, et al. Complications of total implantable access ports and efficacy of Taurolidine-citrate lock solution against catheter-related infections. Afr J Paediatr Surg. 2014;11(2):138-42.
48. Simon A, Ammann RA, Wiszniewsky G, Bode U, Fleischhack G, Besuden MM. Taurolidine-citrate lock solution (TauroLock) significantly reduces CVAD-associated grampositive infections in pediatric cancer patients. BMC Infect Dis. 2008;8:102.
49. Clark J, Graham N, Kleidon T, Ullman A. Taurolidine-Citrate Line Locks Prevent Recurrent Central Line Associated Bloodstream Infection in Pediatric Patients. Pediatr Infect Dis J. 2018.
50. Chong CY, Ong RY, Seah VX, Tan NW, Chan MY, Soh SY, et al. Taurolidine-citrate lock solution for the prevention of central line-associated bloodstream infection in paediatric haematology-oncology and gastrointestinal failure patients with high baseline central-line associated bloodstream infection rates. J Paediatr Child Health. 2020;56(1):123-9.
51. Marschall J, Mermel LA, Fakih M, Hadaway L, Kallen A, O'Grady NP, et al. Strategies to prevent central line-associated bloodstream infections in acute care hospitals: 2014 update. Infect Control Hosp Epidemiol. 2014;35 Suppl 2:S89-107.
52. prevention Cfdca. 2017 Recommendations on use of chlorhexidine-impregnated dressings for prevention of intravascular catheter-related infections: An update to the 2011 guidelines for the prevention of intravascular catheter-related infections from the Centers for Disease Control and Prevention.: Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Division of Healthcare Quality and Promotion.; 2017.
53. van de Wetering MD, van Woensel JB, Lawrie TA. Prophylactic antibiotics for preventing Gram positive infections associated with long-term central venous catheters in oncology patients. Cochrane Database Syst Rev. 2013(11):CD003295.
54. Ferreira Chacon JM, Hato de Almeida E, de Lourdes Simoes R, Lazzarin COV, Alves BC, Mello de Andrea ML, et al. Randomized study of minocycline and edetic acid as a locking solution for central line (port-a-cath) in children with cancer. Chemotherapy. 2011;57(4):285-91.

55. Chatzinikolaou I, Zipf TF, Hanna H, Umphrey J, Roberts WM, Sherertz R, et al. Minocycline-ethylenediaminetetraacetate lock solution for the prevention of implantable port infections in children with cancer. Clin Infect Dis. 2003;36(1):116-9.
56. Wolf J, Connell TG, Allison KJ, Tang L, Richardson J, Branum K, et al. Treatment and secondary prophylaxis with ethanol lock therapy for central line-associated bloodstream infection in paediatric cancer: a randomised, double-blind, controlled trial. Lancet Infect Dis. 2018;18(8):854-63.
57. Longo R, Llorens M, Goetz C, Platini C, Eid N, Sellies J, et al. Taurolidine/Citrate Lock Therapy for Primary Prevention of Catheter-Related Infections in Cancer Patients: Results of a Prospective, Randomized, Phase IV Trial (ATAPAC). Oncology. 2017.
58. Shah CB, Mittelman MW, Costerton JW, Parenteau S, Pelak M, Arsenault R, et al. Antimicrobial activity of a novel catheter lock solution. Antimicrob Agents Chemother. 2002;46(6):1674-9.
59. Torres-Viera C, Thauvin-Eliopoulos C, Souli M, DeGirolami P, Farris MG, Wennersten CB, et al. Activities of taurolidine in vitro and in experimental enterococcal endocarditis. Antimicrob Agents Chemother. 2000;44(6):1720-4.
60. Traub WH, Leonhard B, Bauer D. Taurolidine: in vitro activity against multiple-antibiotic-resistant, nosocomially significant clinical isolates of Staphylococcus aureus, Enterococcus faecium, and diverse Enterobacteriaceae. Chemotherapy. 1993;39(5):322-30.
61. Olthof ED, Nijland R, Gulich AF, Wanten GJ. Microbiocidal effects of various taurolidine containing catheter lock solutions. Clin Nutr. 2015;34(2):309-14.
62. Olthof ED, Rentenaar RJ, Rijs AJ, Wanten GJ. Absence of microbial adaptation to taurolidine in patients on home parenteral nutrition who develop catheter related bloodstream infections and use taurolidine locks. Clin Nutr. 2013;32(4):538-42.
63. Fahrner R, Moller A, Press AT, Kortgen A, Kiehntopf M, Rauchfuss F, et al. Short-term treatment with taurolidine is associated with liver injury. BMC Pharmacol Toxicol. 2017;18(1):61.
64. GMBH T. TauroLock™ Antimicrobial Catheter Lock System to Provide Patency and Infection Control TauroLock™ Brochure [Available from: http://www.taurolock.com/sites/default/files/downloadsTauroLock_Prospekt_85100-20-16_web.pdf.
65. Goossens GA. Flushing and Locking of Venous Catheters: Available Evidence and Evidence Deficit. Nurs Res Pract. 2015;2015:985686.
66. Chow SC, Shao J, Wang H. Sample Size Calculations in Clinical Research. Second Edition ed. Boca Raton, Florida.: Chapman & Hall/CRC; 2008.
67. D'Agostino RB, Chase W, Belanger A. The Appropriateness of Some Common Procedures for Testing the Equality of Two Independent Binomial Populations. The American Statistician. 1988;42(3):198-202.
68. Fleiss JL, Levin B, Paik MC. Statistical Methods for Rates and Proportions. Third Edition. ed. New York: John Wiley & Sons; 2003.
69. Machin D, Campbell M, Fayers P, Pinol A. Sample Size Tables for Clinical Studies. Second Edition ed. Malden, Mass: Blackwell Science; 1997.
70. Ryan TP. Sample Size Determination and Power. Hoboken, New Jersey.: John Wiley & Sons; 2013.

71. Lachin JM. Biostatistical Methods. . New York: John Wiley & Sons; 2000.
72. FDA. February 2017 510(k) Clearances: U.S. Food and Drug Administration (FDA); 2017 [Available from: https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/510kClearances/ucm544999.htm.
73. FDA. https://www.accessdata.fda.gov/cdrh_docs/pdf16/K163591.pdf: U.S. Food and Drugs Administration (FDA) [
74. FDA. Heparin Catheter Lock-Flush Solutions; Transfer of Primary Responsibility from Center for Drug Evaluation and Research to Center for Devices and Radiological Health. In: services Dohah, editor. Federal Register2006. p. 47499-500.
75. Luther MK, Mermel LA, LaPlante KL. Comparison of ML8-X10 (a prototype oil-in-water micro-emulsion based on a novel free fatty acid), taurolidine/citrate/heparin and vancomycin/heparin antimicrobial lock solutions in the eradication of biofilm-producing staphylococci from central venous catheters. J Antimicrob Chemother. 2014;69(12):3263-7.
76. Schoot RA, van de Wetering MD, Stijnen T, Tissing WJ, Michiels E, Abbink FC, et al. Prevalence of Symptomatic and Asymptomatic Thrombosis in Pediatric Oncology Patients With Tunneled Central Venous Catheters. Pediatr Blood Cancer. 2016;63(8):1438-44.
77. Conway MA, McCollom C, Bannon C. Central Venous Catheter Flushing Recommendations: A Systematic Evidence-Based Practice Review. J Pediatr Oncol Nurs. 2014;31(4):185-90.
78. MK B. Taurolin. Ein neues Konzept zur antimikrobiellen Chemothe- rapie chirurgischer Infektionen. München: Urban & Schwarzenberg; 1985.
79. TauroSept G. Product Monograph: The Medical Solution for Treatment and Prevention of Catheter Infections [Available from: http://www.versium.sk/wp-content/uploads/2015/04/Taurosept-Monograph.pdf.
80. Services USDoHaH. Common Terminology Criteria for Adverse Events (CTCAE) 2017 [Available from: https://www.eortc.be/services/doc/ctc/CTCAE_v5_Quick_Reference_5x7.pdf.
81. National Healthcare Safety Network (NHSN) device associated module 2022 [Available from: https://www.cdc.gov/nhsn/pdfs/pscmanual/4psc_clabscurrent.pdf.
82. Tomlinson D, Mermel LA, Ethier MC, Matlow A, Gillmeister B, Sung L. Defining bloodstream infections related to central venous catheters in patients with cancer: a systematic review. Clin Infect Dis. 2011;53(7):697-710.
83. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309-32.
84. See I, Soe MM, Epstein L, Edwards JR, Magill SS, Thompson ND. Impact of removing mucosal barrier injury laboratory-confirmed bloodstream infections from central line-associated bloodstream infection rates in the National Healthcare Safety Network, 2014. Am J Infect Control. 2017;45(3):321-3.
85. Martin D. AH. An efficient program for computing conditional maximum likelihood estimates and exact confidence limits for a common odds ratio. Epidemiology 1991;2:359-62.
86. Martin D. AH. Exact estimates for a rate ratio. Epidemiology 1996;7:29-33.
87. Putter H, Fiocco M, Geskus RB. Tutorial in biostatistics: competing risks and multi-state models. Stat Med. 2007;26(11):2389-430.

88. Gray RJ. A Class of K-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk. The Annals of Statistics. 1988;16(3):1141-54.
89. van Houwelingen HC. Dynamic Prediction by Landmarking in Event History Analysis. Scandinavian Journal of Statistics. 2006;34(1):70-85.