CLINICAL STUDY REPORT

1 TITLE PAGE

Study title:	A Prospective Randomized Double Blind Multicenter Phase III Study Comparing two Methods of Cardioplegia in Coronary Artery Bypass Surgery Custodiol-N versus Custodiol
Investigational medicinal product:	Custodiol-N
Indication:	Coronary artery bypass surgery
Study design:	Prospective, randomized, double-blind, active comparator, multicenter Phase III study
Study dates:	19 May 2011 – 09 March 2012
Development phase:	Phase III
Sponsor's name and address:	Dr. F. Köhler Chemie GmbH Werner-von-Siemens-Str. 22 - 28 D-64625 Bensheim, Germany
Study number:	CL-N-CSM-III/01/08
EudraCT number	2008-005992-81
Investigator(s):	Co-ordinating Investigator: Prof. Dr. G. B. Szabó Department of Cardiac Surgery University of Heidelberg Im Neuenheimer Feld 110 D-69120 Heidelberg, Germany Phone: +49 - 6221 – 566 111 Fax: +49 - 6221 – 565 585 Refer to Section 16.1.4 for names and addresses of further investigators.
GCP compliance statement:	Refer to Sections 4.1.2 and 7.6 of the report.
Date:	25 Feb 2013 final
Confidentiality statement:	**This report is confidential. It may not be used for any purpose without the prior written permission of the Sponsor of this study.**

I have read this report and confirm that, to the best of my knowledge, it accurately describes the conduct and results of the study:

Title:

A Prospective Randomized Double Blind Multicenter Phase III Study Comparing two Methods of Cardioplegia in Coronary Artery Bypass Surgery Custodiol-N versus Custodiol

Study number: CL-N-CSM-III/01/08

Prof. Dr. med. Gábor B. Szabó	Role: Co-ordinating Investigator
Date:	Signature:
Dr. med. Roman Petrov	Role: Sponsor Signatory
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Dr. rer. medic. Johannes Hüsing	Role: Biometrician
Date:	Signature:

2 SYNOPSIS

Title of the study:	A Prospective Randomized Double Blind Multicenter Phase III Study Comparing two Methods of Cardioplegia in Coronary Artery Bypass Surgery Custodiol-N versus Custodiol Study No.: CL-N-CSM-III/01/08
Co-ordinating Investigator:	Prof. Dr. G. B. Szabó Department of Cardiac Surgery University of Heidelberg Im Neuenheimer Feld 110 D-69120 Heidelberg, Germany Phone: +49 - 6221 – 566 111 Fax: +49 - 6221 – 565 585
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	Klinik für Thorax- und Kardiovaskuläre Chirurgie Universitätsklinikum Essen Hufelandstraße 55 45147 Essen Phone: +49 - 2 01/ 7 23 – 0 Fax: +49 - 2 01/ 7 23 – 46 No patients were enrolled in Essen.
Publications (references):	Not applicable.
Period of study:	19 May 2011 – 09 March 2012
Clinical phase:	Phase III
Objectives:	The objective of this study was to compare the cardioprotective effects and safety of 2 cardioplegic solutions, HTK Cardioplegic Solution (Custodiol®) and Custodiol-N in patients undergoing cardiopulmonary bypass for coronary artery bypass surgery.
Methodology (design of study):	Prospective, randomized, double blind, multicenter Phase III comparison study intended to demonstrate non-inferiority in surgical outcome between Custodiol® and Custodiol-N as determined by CK-MB area under the curve (AUC; primary endpoint), catecholamine requirement (cumulative dose), cardiac Troponin T and occurrence of comorbid events postoperatively (e.g., myocardial infarction). The duration of the study for each patient was expected to be 6 to 7 weeks. The study population was selected from patients of either sex with coronary artery disease (CAD) who were to undergo cardiopulmonary bypass for coronary artery bypass surgery.
Number of patients:	Ten to 516 patients were planned to be enrolled in order to yield a final sample size of up to 455 patients completing the study. Recruitment took place in 3 stages. In the first stage, 10 patients were administered Custodiol-N and evaluated for safety ("Custodiol-N only treated cohort"). These patients were included in the final analysis of safety but not in the efficacy analysis. In the second stage, 50 patients were

<table>
<tr><td></td><td>enrolled. A planned interim analysis in these patients showed results which would have been sufficient to show non-inferiority of Custodiol-N compared to Custodiol® at the planned 30% margin. Thus, it was decided only to continue recruitment in the third stage until the sample size of 100 patients considered necessary for safety analyses had been reached.

Table 1: Number of patients in the study
<table>
<tr><th></th><th>Custodiol-N</th><th>Custodiol®</th><th>Total</th></tr>
<tr><td>Enrolled</td><td>60</td><td>52</td><td>112</td></tr>
<tr><td>Treated</td><td>59</td><td>52</td><td>111</td></tr>
<tr><td>Safety set</td><td>59</td><td>52</td><td>111</td></tr>
<tr><td>Full analysis set (FAS)</td><td>49</td><td>52</td><td>101</td></tr>
<tr><td>Per-protocol (PP) set</td><td>43</td><td>44</td><td>87</td></tr>
</table></td></tr>
<tr><td>Diagnosis and main criteria for inclusion:</td><td><u>Key inclusion criteria</u>: Male or female patients ≥ 35 and ≤ 80 years of age with 2 or 3 vessel coronary disease who were scheduled for elective CBP surgery for coronary revascularization; presence of definite CAD for which surgical intervention was deemed necessary, without evidence of ongoing infarction; eligibility for Swan-Ganz-Catheter.

<u>Key exclusion criteria</u>: Patients undergoing valve repair or replacement; history of recent (< 6 weeks) Q-wave myocardial infarction; left ventricular ejection fraction < 35%; patients on intra-aortic balloon devices or with history of previous coronary artery bypass surgery; patients in cardiogenic shock, with severe chronic obstructive lung disease, with previous clinically relevant cardiac valvular disease, on dialysis or with creatinine > 2 mmol/L; with bare metal stent < 4 weeks, with drug-eluting stent < 6 months or with guidance dependent Plavix® therapy</td></tr>
<tr><td>Test product, dose and mode of administration, batch number:</td><td>The study drug was Custodiol-N. After cross clamping of the aorta on cardiopulmonary bypass, the Custodiol-N solution, at a temperature of 4 - 6°C, was infused antegrade into the root of the aorta. The infusion was continued for 7 minutes independent of the total volume of 1000-2000 mL.</td></tr>
</table>

	The following batches were used: Nos. 1108321 (Custodiol-N only treated cohort), 1201231, 1129831, 1123031
Duration of treatment:	Not applicable.
Reference therapy, dose and mode of administration, batch number:	The active comparator was Custodiol®. After cross clamping of the aorta on cardiopulmonary bypass, the Custodiol® solution, at a temperature of 4 - 6°C, was infused antegrade into the root of the aorta. The infusion was continued for 7 minutes independent of the total volume of 1000-2000 mL. The following batches were used: Nos. 1201111, 1129912, 1122911
Criteria of evaluation:	The primary endpoint was the creatine kinase muscle-brain (CK-MB) AUC within 24 hours (measurements 4, 8, 12, 16, 20, 24 hours ± 30 min) after release of the aortic cross clamp. Secondary endpoints were: – Catecholamine requirement on surgical intensive care unit (SICU) within 24 hours (cumulative dose) – CK-MB (peak) on the days 2, 3, 4 and 5 after removal of aortic cross clamp – Cardiac troponin T 4,8,12,16,20,24 hours ± 30 min and on the days 2, 3, 4 and 5 after release of the aortic cross clamp – Cardiac index – Defibrillation – Requirement for intraaortic balloon pump (IABP) – Blood pressure – Length of SICU stay – Duration of mechanical ventilation (intubation to extubation) – Pulmonary and systemic vascular resistance – Occurrence, severity, type, and duration of cardiac arrhythmias – Laboratory parameters – Repeated patient transfer to SICU

	– Mortality any time during post-op through Day 30 – Safety (documentation and reporting of adverse events [AEs] and serious adverse events [SAEs])
Statistical methods:	The $AUC_{4\text{-}24h}$ for CK-MB was derived from the single measurements using the trapezoidal method on the scheduled time points. For modeling purposes the AUC was transformed by natural logarithm, as the parameter is known to have a right-skewed distribution and for a more natural approach to relative differences. Both, the FAS and the PP set were used to answer the study question; the null hypothesis was: H_0: The AUC for CK-MB in the Custodiol-N treatment group exceeds, for the FAS/PP set, the AUC for CK-MB in the Custodiol® treatment group by at least 30%. The test statistic (Z_F for the FAS and Z_P for the PP set) was the difference of the means of logarithms of the AUC values between the Custodiol-N and Custodiol® treatment groups, added to the logarithm of 130%, and divided by the common standard error of the logarithm of the AUC. The $AUC_{4\text{-}24h}$ for troponin T was determined in the same manner as for the primary endpoint: The log AUC (obtained by the trapezoidal rule) was averaged in either treatment group; the difference of the means was divided in order to obtain a t-statistic to calculate a 95% confidence interval (CI) for the relative difference between treatments. Further secondary endpoints were analyzed, as appropriate, using descriptive summary statistics or by tabulation of absolute and relative frequencies. AEs were coded according to MedDRA. After the analysis of 50 patients, an estimation of the sample size necessary to answer the study hypothesis was planned. If either one of Z_F and Z_P was below the critical value of $u_{1-\alpha_1} \approx -0.5244$ (where u was the quantile of the standard normal distribution and α_1 was set to the futility threshold of 70%), the study would have had to be stopped for futility.

Summary and conclusions:

Summary of efficacy:

A total of 112 patients (60 in the Custodiol-N group and 52 in the Custodiol® group) were enrolled in 4 study centers. One patient in the Custodiol-N group did not receive study treatment and was excluded from all analysis populations. All other patients were treated. Thus, the safety population comprised 59 patients in the Custodiol-N group and 52 patients in the Custodiol® group. Since the patients from the "Custodiol-N only treated cohort" were excluded from efficacy analyses, the FAS comprised 49 patients in the Custodiol-N group and 52 patients in the Custodiol® group. Six patients in the Custodiol-N group and 8 patients in the Custodiol® group were excluded from the PP set due to protocol deviations, which thus comprised 43 patients in the Custodiol-N group and 44 patients in the Custodiol® group. There was an imbalance in the number of patients enrolled in the 4 study centers with a preponderance of patients included in Heidelberg (FAS: 82 patients in Heidelberg and 19 patients in all other sites).

Apart from higher rates of tobacco abuse, angina pectoris and hypercholesterolemia in the Custodiol-N group, demography and baseline characteristics were overall comparable between the treatment groups. Approximately 80% of patients were men. The mean age was 68.1 ± 8.0 years in the Custodiol-N group and 66.4 ± 8.5 years in the Custodiol® group. The most common NYHA class was II (49.0% of patients in the Custodiol-N group and 51.9% in the Custodiol® group); the mean left ventricular ejection fraction was 62.5 ± 8.1% in the Custodiol-N group and 59.3 ± 10.5% in the Custodiol® group. The median cross clamp time was 41 minutes in the Custodiol-N group and 47 minutes in the Custodiol® group.

Primary efficacy results are summarized in Table 2.

Table 2: Primary analysis: Treatment effect on CK-MB AUC_{4-24h}

Parameter	**% Difference** (Custodiol® minus Custodiol-N)[a]	**[95% CI]**	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 30%)	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 0%)	**Analysis set**
CK-MB	2.7	[-8.3; 15.0]	<0.0001	0.3192	FAS
	2.5	[-9.2; 15.8]	<0.0001	0.3427	PP set

[a] = Antilog of difference of mean log(AUC) in Custodiol® patients minus mean log(AUC) in Custodiol-N patients.
Abbreviations: CI = Confidence interval; FAS = Full analysis set; H_0 = Null hypothesis; PP = Per-protocol

The primary analysis statistically confirmed non-inferiority of Custodiol-N compared with Custodiol® as determined by the CK-MB AUC_{4-24h} ($p< 0.0001$ at the 30% non-inferiority margin both in the FAS and in the PP set). The mean CK-MB AUC_{4-24h} was 779 ± 439 (median: 620; range: 270-2672) h*U/L in the Custodiol-N group and 878 ± 549 (median:

742; range: 100-2550) h*U/L in the Custodiol® group. The result of the primary analysis was supported by the results of the corresponding secondary analyses of troponin T $AUC_{4\text{-}24h}$, and CK-MB and troponin T peak values as summarized in Table 3. For CK-MB peak values, evaluation at the 0% margin (p=0.032 in the FAS; p=0.0782 in the PP set) pointed towards more favorable results under Custodiol-N.

Table 3: Secondary endpoints: Treatment effect on troponin T $AUC_{4\text{-}24h}$, CK-MB and troponin T peak values

Parameter	**% Difference** (Custodiol® minus Custodiol-N)	**[95% CI]**	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 30%)	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 0%)	**Analysis set**
Troponin T	-9.2 [a]	[-19.1; 1.9]	0.0027	0.9495	FAS
	-7.7 [a]	[-18.5; 4.6]	0.0022	0.8966	PP set
CKMB peak	10.9	[-0.6; 23.9]	<0.0001	0.0322	FAS
	9.2	[-3.4; 23.3]	<0.0001	0.0782	PP set
Troponin T peak	-3.0	[-13.0; 8.2]	<0.0001	0.7075	FAS
	-0.2	[-11.4; 12.3]	<0.0001	0.5143	PP set

Note: Secondary analysis → P-values have to be interpreted descriptively.
[a] = Antilog of difference of mean log(AUC) in Custodiol® patients minus mean log(AUC) in Custodiol-N patients.
Abbreviations: CI = Confidence interval; FAS = Full analysis set; H_0 = Null hypothesis; PP = Per-protocol

There was an imbalance in the number of patients enrolled in the 4 study centers with a preponderance of patients included in Heidelberg (FAS: 82 patients in Heidelberg and 19 patients in all other sites). A subgroup analysis differentiated by study center showed a high variability of values in the "all other sites" subgroup. The distance to the non-inferiority margin for CK-MB $AUC_{4\text{-}24h}$ was more pronounced in the Heidelberg subgroup (36.0%, p<0.0001) than in the "all other sites" subgroup (28.0%, p=0.1705) (FAS). The same was observed for the secondary endpoint troponin T, with a distance to the 30% non-inferiority margin of 24.6% (p=0.0009) in the Heidelberg subgroup and 17.3% (p=0.2937) in the "all other sites" subgroup.

Results of further secondary endpoints are summarized in Table 4.

Table 4: Further secondary endpoints

			Custodiol-N	Custodiol®
CKMB peak values [U/L]		N	45	48
		Mean ± SD	49 ± 30	58 ± 40
Troponin T peak values [pg/mL]		N	49	52
		Mean ± SD	946 ± 471	931 ± 554
Patients who received catecholamines*		N	49	52
		n (%)	46 (93.9)	45 (86.5)
Cardiac index [L/min/m²]	Baseline	N	46	50
		Mean ± SD	2.1 ± 0.4	2.2 ± 0.5
	at 4 hours	N	40	41
		Mean ± SD	2.4 ± 0.7	2.9 ± 0.7
	at 24 hours	N	39	33
		Mean ± SD	2.8 ± 0.7	3.0 ± 0.6
No. of patients with postoperative defibrillation			0	1
No. of patients with intraaortic balloon pump			0	0
Systolic blood pressure [mmHg]	Baseline	N	49	51
		Mean ± SD	139 ± 20	138 ± 22
	Day 5	N	49	51
		Mean ± SD	131 ± 17	132 ± 20
Diastolic pressure [mmHg]	Baseline	N	49	51
		Mean ± SD	76 ± 13	77 ± 11
	Day 5	N	49	49
		Mean ± SD	72 ± 12	76 ± 15
Day of discharge from intensive care unit, study day		N	49	50
		Mean ± SD	3 ± 2	3 ± 5
Duration of mechanical ventilation, hours**		N	48	49
		Mean ± SD	25 ± 17	21 ± 17
Pulmonary vascular resistance [dyn*s/cm⁵]	At 4 hours§	N	39	43
		Mean ± SD	185 ± 147	157 ± 118
	At 24 hours#	N	39	30
		Mean ± SD	145 ± 87	134 ± 77
Systemic vascular resistance [dyn*s/cm⁵]	At 4 hours	N	45	43
		Mean ± SD	1316 ± 811	1044 ± 459
	At 24 hours	N	40	36
		Mean ± SD	1210 ± 399	1025 ± 358
Patients with postoperative atrial fibrillation		N	49	51
		n (%)	14 (28.6)	16 (31.4)
Patients with postoperative ventricular tachycardia		N	49	51
		n (%)	4 (8.2)	1 (2.0)
Patients with postoperative ventricular fibrillation		N	49	51
		n (%)	0	1 (2.0)
Patients re-admitted to the intensive care unit within 5 days			0	1
Deaths through Day 30[a]			1	1

* = p=0.12; Mantel-Haenszel Chi-Square test
** = p=0.070; Wilcoxon rank sum test
§= p=0.32; Wilcoxon rank sum test
#= p=0.50; Wilcoxon rank sum test
[a] = One further patient in the Custodiol® group died on day 43.

Summary of safety:

A total of 79.7% of patients in the Custodiol-N group and 86.5% of patients in the Custodiol® group experienced AEs. Most frequently reported were "cardiac disorders" (47.5% in the Custodiol-N group and 44.2% in the Custodiol® group) and "injury, poisoning & procedural complications" (20.3% in the Custodiol-N group and 28.8% in the Custodiol® group). Most common AEs were atrial fibrillation (28.8% in the Custodiol-N group and 30.8% in the Custodiol® group) and anemia (13.6% in the Custodiol-N group and 11.5% in the Custodiol® group). One case of acute myocardial infarction (original term: N-STEMI [non-ST elevation myocardial infarction]) was reported in the Custodiol® group. The event was considered not to be related to the study medication and the outcome was "recovered". One case of cardiac arrest was reported in each treatment group. One of the cases (Custodiol® group) was fatal; the other patient (Custodiol-N group) recovered. Neither case was considered to be related to the study medication. The majority of AEs was considered not to be related to the study medication. Drug-related AEs (specified as probably related) were reported in 1 patient (1.7%) in the Custodiol-N group (atrial fibrillation and pleural effusion) and in 2 patients (3.8%) in the Custodiol® group (1 patient with psychotic disorder and 1 patient with 2 events of atrial fibrillation and 1 event of respiratory failure). The majority of patients experienced AEs of maximally mild or moderate severity. Severe AEs were reported in 4 patients (6.8%) in the Custodiol-N group (gamma-glutamyltransferase increased, anesthetic complication neurological, ventricular fibrillation and cardiac arrest) and in 2 patients (3.8%) in the Custodiol® group (pneumothorax and skin emphysema in 1 patient and cardiac arrest in another patient). SAEs were reported for 1 patient in the Custodiol-N group (cardiac arrest, outcome: recovered) and for 3 patients in the Custodiol® group (pleural effusion [outcome: recovered], pneumothorax [outcome: fatal] and cardiac arrest [outcome: fatal]). None of the SAEs was considered as being related to the study medication. Three deaths (1 in the Custodiol-N group and 2 in the Custodiol®) group were reported, one of which occurred outside of the follow-up period on Day 43 (cause of death: severe respiratory insufficiency due to progressive pneumothorax; Custodiol® group). One patient in the Custodiol® group died of cardiac arrest on Day 0 and 1 patient in the Custodiol-N group died of ventricular fibrillation on Day 7 (outside of the reporting period for AEs/SAEs which was up to Day 5). Evaluation of laboratory parameters showed changes which can be expected following cardiac surgery and cardiopulmonary bypass; no relevant differences between the treatment groups were observed.

General Conclusions:

In conclusion, non-inferiority of Custodiol-N compared with Custodiol® with respect to their cardioprotective effect in patients undergoing cardiopulmonary bypass for coronary artery bypass surgery was statistically confirmed based on CK-MB $AUC_{4\text{-}24h}$ as marker for myocardial damage. The safety analysis showed comparable results for both drugs.

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3 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	Adverse event
AUC	Area under the curve
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte (Federal Institute for Drugs and Medical Devices)
bpm	Beats per minute
BSA	Body surface area
CABG	Coronary artery bypass graft
CAD	Coronary artery disease
CI	Confidence interval
eCRF	electronic Case report form
FAS	Full analysis set
FEV	Forced expiratory volume
GCP	Good clinical practice
GGT	Gamma-glutamyl transferase
HTK	Histidine-tryptophan-ketoglutarate
IABP	Intraaortic balloon pump
ICU	Intensive care unit
IEC	Independent Ethics Committee
MedDRA	Medical Dictionary for Regulatory Activities
NYHA	New York Heart Association
mEq/L	Milliequivalents per liter
PP	Per-protocol
PT	Preferred Term
Q1	First quarter
SICU	Surgical intensive care unit
SOC	System Organ Class
SAE	Serious adverse event
TnT	Troponin T

4 ETHICS, INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

4.1 Ethics

4.1.1 Independent Ethics Committee (IEC) or Institutional Review Board (IRB)

Before the start of the study, the study protocol, informed consent document, and any other appropriate documents were submitted to the Independent Ethics Committee (IEC) as well as to the competent higher federal authority (BfArM). A written favorable vote of the IEC and an (implicit) approval by the competent higher federal authority were a prerequisite for initiation of this clinical study.

Before the first patient was enrolled in the study, all ethical and legal requirements were met. All planned substantial changes (see §10, (1) of German Good Clinical Practice [GCP]-Regulation) were submitted to the IEC and to the competent higher federal authority in writing as protocol amendment which was approved by the IEC and the competent higher federal authority.

Please refer to Section 16.1.3.1 for a list of IECs.

4.1.2 Ethical Conduct of the Study

The procedures set out in the study protocol, pertaining to the conduct, evaluation, and documentation of this study, were designed to ensure that all persons involved in the study abided by GCP and the ethical principles described in the applicable version of the Declaration of Helsinki. The study was carried out in keeping with local legal and regulatory requirements.

4.1.3 Patient Information and Informed Consent

Before being admitted to this study, the patient was to consent to participate after the nature, scope, and possible consequences of the study had been explained in a form understandable to him or her. The patient was to give consent in writing. The signed Informed Consent Form was filed by the investigator.

A copy of the signed informed consent document was given to the patient. The document was provided in a language understandable to the patient and specified who informed the patient.

The patients were to be informed as soon as possible if new information might have influenced his/her decision to participate in the study. The communication of this information should be documented.

A copy of the Informed Consent Form and the Patient Information are provided in Section 16.1.3.2.

4.2 Investigators and Study Administrative Structure

Co-ordinating Investigator:	Prof. Dr. G. B. med. Szabó Department of Cardiac Surgery University of Heidelberg Im Neuenheimer Feld 110 D-69120 Heidelberg Germany
Sponsor:	Dr. med. (RUS) Roman Petrov Head Clinical Research Dr. F. Köhler Chemie GmbH Werner-von-Siemens-Str. 22 - 28 D-64625 Bensheim Germany
Biometrician:	Dr. rer. medic. Johannes Hüsing Qualified Statistician Head of Biometrics Koordinierungszentrum für Klinische Studien (KKS) University of Heidelberg Voßstraße 2 / Gebäude 4410 D-69115 Heidelberg Germany
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Medical Writing:	Winicker Norimed GmbH Clinical Research Deutschherrnstraße 15-19 D-90429 Nürnberg
Data Safety Monitoring Board (DSMB):	Prof. Dr. med. Ali El-Armouche Department of Pharmacology Georg-August University Göttingen Robert-Koch-Str. 40 D-37075 Göttingen Germany Prof. Dr. med. Christian Hagl Director Heart Surgery Clinic and Polyclinic University Hospital Munich Marchioninistraße 15 D-81377 Munich Germany Stefan Bruns Head Biometry and Data Management Winicker Norimed GmbH Medizinische Forschung Deutschherrnstraße 15-19 90429 Nürnberg

A complete list of all investigators and other important participants in the study is provided Section 16.1.4.

5 INTRODUCTION

5.1 Cardioplegia

Cardioplegia, the procedure by which the heart is arrested in preparation for surgery, is typically performed via injection of a cold crystalloid or blood-based solution into the root of the aorta. The aim of this technique is to arrest the heart (maintain cardiac standstill) and to induce uniform hypothermia, which protects the myocardium during the operative period.

Empirical observations have revealed that after approximately 90 minutes of cardiac arrest, irreversible ischemia occurs and the patient sustains myocardial injury (indicated by cellular leak of cardiac enzymes). Maintaining the heart at low temperatures (10-15 °C) decreases the oxygen and energy requirements, but the longer the period of ischemia, the greater the likelihood of permanent myocardial damage. When cardioplegic solutions are used, the heart is eventually reperfused with the systemic blood of the patient by releasing the aortic clamp. Several events, however, may occur during the reperfusion period which may induce some additional degree of damage to the myocardium.

Although most patients recover using current techniques, morbidity and mortality still occur in patients with marginal left ventricular function, patients with recent or extensive myocardial infarctions, and patients with hypertrophic hearts. These concerns have prompted the development of improved methods for myocardial preservation. Cold blood cardioplegia (1,2,3,4,5,6) replaces the clear crystalloid cardioplegic solution with a mixture of blood and highly concentrated potassium solution. Additional agents (such as bicarbonate) are added to serve as a buffering agent against the acidosis which develops in the ischemic organ. Even with this method, patients with acute myocardial infarction or marginal cardiac reserve still show increased morbidity and mortality.

5.2 Custodiol® (histidine-tryptophan-ketoglutarate, HTK solution)

Custodiol® (HTK cardioplegic solution) has a low sodium content (which induces cardiac arrest) making it possible to use a high concentration of biological buffer systems, namely histidine, histidine hydrochloride and tryptophan, which confer a buffering capacity superior to that of blood (7,8,9,10). This combination markedly delays the development of myocardial acidosis and significantly improves the provision of adenosine triphosphate (ATP) by an enhanced degradation of glycogen to lactate during ischemia (11,12,13). A one-time only infusion of approximately 4 liters is protective for a period of up to 3 hours of ischemia (8). Since HTK solution is clear (does not contain blood) it does not obscure the operative field

for coronary artery bypass grafts (e.g., CABG) or heart valve replacements. At the end of that time, the heart is reperfused by the systemic blood and recovers its normal function within minutes.

Experimental evidence in both animals and humans supporting the mechanism by which HTK solution works is convincing and suggests that, in comparison with other currently available solutions, HTK may offer superior protection (7,8,12). The solution has been used for more than 25 years in tens of thousands of cases throughout Europe (14).

5.3 Custodiol-N

Custodiol-N is an amino acid-fortified and iron chelator-supplemented cardioplegic and organ preservation solution that is (otherwise) based on the principles of Custodiol® solution.

It has been shown in several cell types that the amino acids glycine and alanine provide protection against hypoxic or ischemic cell injury (15,16 ,17 ,18 ,19 ,20 ,21). Furthermore, it became clear that hypothermia, used to protect tissues against ischemic injury, also triggers injury on its own account (4,5,6,22,23); this cold-induced cell injury affects numerous cell types but in particular endothelial cells, jeopardizing vascular function after reperfusion, and is mediated by intracellular "redox-active" iron (6,11,7,8). Based on these experimental findings traditional Custodiol® solution was fortified with the amino acids glycine and alanine and was supplemented by the strong, but poorly membrane-permeable iron chelator deferoxamine and the new, membrane-permeable iron chelator LK 614.

Furthermore, recent studies have shown that the buffer histidine, essential for the efficiency of HTK solution, can have adverse effects on some cell types rich in "redox-active" iron, particularly, if used at very high concentrations (9). The histidine derivative N-acetyl-histidine does not display these adverse effects but has a similar buffering power as histidine. Therefore, part of the histidine in Custodiol® solution (as much as possible without altering the successful ion composition of the solution) was replaced by the superior derivative N-acetyl-histidine.

Custodiol-N has been supplemented with L-arginine which proved to decrease microcirculatory disturbances (10,12,13,24). As mannitol is not impermeable to all cell types (esp. hepatocytes (25)), it has been replaced by saccharose. Although efficient buffering is important to prevent severe acidosis, moderate acidosis has been shown to provide protection against ischemic injury (26,27); therefore Custodiol-N has a slightly lower pH than the traditional Custodiol® solution. Finally, aspartate has been added to allow, in combination with the α-ketoglutarate already present in Custodiol®, the replenishment of intermediates of

the tricarboxylic acid cycle and thus efficient energy production after reperfusion. Besides these alterations, the successful composition of Custodiol® has been maintained.

Custodiol-N proved to be superior to traditional Custodiol® solution in in vitro studies using diverse cell types, in particular, Custodiol-N showed far superior inhibition of hypoxic cell injury, superior to far superior inhibition of cold-induced cell injury and avoidance of adverse effects, also during warm exposure to the solution. In a study on the isolated perfused rat heart (Langendorff model) a protective effect of the iron chelators deferoxamine and LK 614, i.e. the iron chelators present in Custodiol-N, on the injury to the coronary vasculature inflicted by cold ischemia could be shown (14). Marked superiority of Custodiol-N over Custodiol® could also be shown in a model of heterotopic heart transplantation in the mouse, in which after 24 hours cold ischemia, 8/8 Custodiol-N-preserved grafts but only 2/8 Custodiol®-preserved grafts were functioning at day 7 post transplantation (K. Wu, O. Witzke, unpublished result).

6 STUDY OBJECTIVES

The objective of this study was to compare the cardioprotective effects and safety of 2 cardioplegic solutions, HTK Cardioplegic Solution (Custodiol®) and Custodiol-N in patients undergoing cardiopulmonary bypass for coronary artery bypass surgery.

7 INVESTIGATIONAL PLAN

7.1 Overall Study Design and Plan-Description

This was a prospective, randomized, double blind, multicenter Phase III comparison study intended to demonstrate non-inferiority in surgical outcome between Custodiol® and Custodiol-N as determined by CK-MB area under the curve (AUC; primary endpoint), catecholamine requirement (cumulative dose), cardiac Troponin T and occurrence of comorbid events postoperatively (e.g., myocardial infarction).

The duration of the study for each patient was expected to be 6 to 7 weeks. The overall duration of the study was expected to be approximately 30 months. The study consisted of 4 study periods: preoperative (baseline), intraoperative, postoperative (in the ICU) and follow-up. The primary hospitalization included part of the preoperative period, the intraoperative period, the postoperative ICU and the follow-up period.

The study population was selected from patients of either sex with coronary artery disease (CAD) who were to undergo cardiopulmonary bypass for coronary artery bypass surgery.

Treatment groups:

Custodiol-N: After cross clamping of the aorta on cardiopulmonary bypass, the Custodiol-N solution, at a temperature of 4 - 6°C, was infused antegrade into the root of the aorta. The infusion was continued for 7 minutes independent of the total volume of 1000-2000 mL of the solution.

Custodiol® (active comparator): After cross clamping of the aorta on cardiopulmonary bypass, the Custodiol® solution, at a temperature of 4 - 6°C, was infused antegrade into the root of the aorta. The infusion was continued for 7 minutes independent of the total volume of 1000-2000 mL of the solution.

Ten to 516 patients were planned to be enrolled in order to yield a final sample size of up to 455 patients completing the study. Recruitment and treatment of patients was planned to be performed in 5 study centers. Further study centers could be selected during the active period of the study. Patients were assigned within the study centers to 1 of 2 treatment groups, Custodiol® or Custodiol-N, using a randomized block design.

Recruitment took place in 3 stages. In the first stage, 10 patients were administered Custodiol-N and evaluated for safety. These patients were not included in the final analysis of efficacy. In the second stage, 50 patients were enrolled. After the second stage, a comparison for efficacy was performed. The study was to stop for futility if the hypothesis of

non-inferiority could not be rejected at the α_1=0.7 level. Otherwise, the study was to continue with at least 50 more patients.

An Independent Data Safety Board (DSMB) was established by the Sponsor. The DSMB was composed of recognized experts in the field of CAD, who are not actively recruiting patients. The mission of the DSMB was to ensure the ethical conduct of the study and to protect the safety interests of patients in this study. The DSMB reviewed relevant data periodically during the study and gave advice on the continuation, modification or termination of the study. The DSMB was unblinded as necessary to perform the interim analysis prepared by the study statistician and issued a recommendation to stop the study for futility or to continue. All SAEs were communicated to and analyzed by the DSMB. The DSMB scrutinized signs and symptoms suggestive of a possible cardiac complication. The DSMB was entitled to issue recommendations to the sponsor and the investigators regarding the continuation or the modification of the study, if there was a suspicion of increased risk of cardiac complications in one of the treatment groups.

7.2 Discussion of Study Design, including the Choice of Control Groups

The study design was appropriate to address the aim of the study.

7.3 Selection of Study Population

The study population was selected from patients of either sex with CAD who were to undergo cardiopulmonary bypass for coronary artery bypass surgery.

7.3.1 Inclusion criteria

1. Patients ≥ 35 and ≤ 80 years of age.
2. Male or female with 2 or 3 vessel coronary disease, who were scheduled for elective CBP surgery for coronary revascularization.
3. Presence of definite CAD for which surgical intervention was deemed necessary, without evidence of ongoing infarction. Patients with unstable angina could be included as long as there was no objective (negative cardiac isoenzymes in the immediate 6 hours preceding CABG, current intravenous use of nitrate therapy) or subjective (absence of prolonged symptoms suggestive of coronary insufficiency that did not respond to pharmacologic intervention) evidence of myocardial necrosis.
4. Eligibility for Swan-Ganz-Catheter.

5. Ability to understand character and individual consequences of the clinical study and to provide written informed consent to participate in the study.
6. No evidence of severe organic or psychiatric disease by history or physical examination.
7. No history of alcohol abuse, illicit drug use, significant mental illness, physical dependence to any opioid, or not any history of drug abuse or addiction within 12 months of study enrollment.

7.3.2 Exclusion criteria

1. Patients undergoing valve repair or replacement.
2. History of recent (< 6 weeks) Q-wave myocardial infarction
3. Left ventricular ejection fraction < 35% (as assessed by any one of the following: contrast ventriculography, multigated acquisition scanning [MUGA], or 2-D ECHO).
4. Patients on intra-aortic balloon devices or with history of previous coronary artery bypass surgery.
5. Pregnant or lactating patients.
6. Patients who had participated in any other investigational studies within 30 days previous to enrollment.
7. Patients in cardiogenic shock (defined as a systolic blood pressure [BP] < 90 mmHg for over 1 hour despite inotropic and chronotropic support).
8. Patients with severe chronic obstructive lung disease (forced expiratory volume in one second [FEV_1] < 50%).
9. Previous cardiac valvular disease (clinically relevant).
10. Dialysis or creatinine > 2 mmol/L.
11. Bare metal stent (BMS) < 4 weeks
12. Drug-eluting stent (DES) < 6 months
13. Guidance dependent Plavix® therapy

7.3.3 Removal of patients from therapy or assessment

Patients who discontinued participation in the clinical study on their own request or patients who were withdrawn by the investigator, for reasons other than disease progression (i.e. in case of AEs, protocol violations, …), were defined as premature withdrawals. Premature withdrawals were not replaced.

In all cases, the reason for withdrawal was to be recorded in the case report form (CRF) and in the patient's medical records. In case of withdrawal of a patient at his/ her own request, the reason should be asked for as extensively as possible and documented. All efforts were to be made to follow up the patient and all examinations scheduled for the final study day were to be performed and documented as far as possible on all patients. For these last examinations the consent of the patient was necessary and was to be requested.

A patient could be / was withdrawn from all study-related procedures (including follow-up visits) for the following reasons:

- at his/her own request or at request of his legal representative
- non-adherence to the study-related requirements, which could (have) influence(d) the validity of the study data.

All ongoing adverse events (AEs) / serious adverse events (SAEs) of withdrawn patients had to be followed up until no more signs and symptoms were verifiable or the patient was in stable condition.

7.4 Treatments

7.4.1 Treatments administered

Routine surgical technique for CABG was employed at all study centers. The body temperature was maintained at 30-32°C. After cross clamping of the aorta on cardiopulmonary bypass, the Custodiol® or Custodiol-N solution, at a temperature of 4 - 6°C, was infused antegrade into the root of the aorta. The method of cannulation (two stage or bicaval) was recorded in the CRF.

The infusion was continued for 7 minutes independent of the total volume of 1000-2000 mL of the solution. In the case of more than 2000 mL – ultrafiltration was suggested. Perfusion pressure in the line should be as usual in each hospital. Throughout the aortic cross clamp period, myocardial temperature was maintained at below 15°C. Simultaneously, the right atrium was opened and the solution exiting the coronary sinus aspirated and discarded via

use of a regular vacuum apparatus. The patient had 2 venous return cannulae in the atrium (28-32 F). A tourniquet was applied around the veins to constrict them over the cannulae in order to bypass completely the right side of the heart. The only fluid in the right atrium was cardioplegic solution coming from the coronary sinus. If the myocardial temperature increased to >15°C, 200 mL of Custodiol® or Custodiol-N was administered.

Occasionally, after complete cardiac standstill was achieved, cardiac activity might again be seen on the ECG monitor or noted by the surgeon. In that case additional infusion of the cardioplegic solution was indicated to sustain cardiac arrest (the volume of infusate varied according to the size of the heart, degree of stenoses, and extent of coronary disease); this volume was recorded in the CRF.

While performing the last coronary anastomosis the patient's body temperature was warmed to reach normothermia (37°C), the aortic clamp was released and the heart re-perfused with systemic blood. Once the cardiac normal rhythm was re-established, the patient was weaned off cardiopulmonary bypass.

7.4.2 Identity of investigational product(s)

The study drug was Custodiol-N. The active comparator was Custodiol®. Custodiol® and Custodiol-N prolong ischemia tolerance principally in two ways:

1. The electrolyte composition of Custodiol® and Custodiol-N blocks the triggering of energy-consuming activation processes. In this way the energy requirements of the organ are reduced to the lowest possible level.
2. Anaerobic energy production is limited by the increasing inhibition of glycolysis due to the drop in pH which results from the accumulation of lactic acid. The histidine/histidine HCl buffer retards the fall in tissue pH during the period of organ ischemia. This elevates the efficiency level of anaerobic glycolytic energy production.

Table 7-1: Composition of Custodiol® and Custodiol-N

	Custodiol®	Custodiol-N
Sodium (mmol/L)	15	16
Potassium (mmol/L)	10	10
Magnesium (mmol/L)	4	8
Calcium (mmol/L)	0.015	0.02
Chloride (mmol/L)	50	30.04
Histidine (mmol/L)	198	124
N-Acetylhistidine (mmol/L)	-	57
Mannitol (mmol/L)	30	-
Sucrose (mmol/L)	-	33
α-Ketoglutarate (mmol/L)	1	1.81
Aspartate (mmol/L)	-	5
Glycine (mmol/L)	-	10
Alanine (mmol/L)	-	5
Tryptophan (mmol/L)	2	2
Arginine (mmol/L)	-	3
Deferoxamine (mmol/L)	-	0.0153
LK 614 (mmol/L)	-	0.0062
pH	7.2	7.0
Osmolarity (mosm/L)	310	305

Data source: Investigator's Brochure

The following batches were used:

Custodiol-N: Nos. 1108321 (Custodiol-N only treated cohort), 1201231, 1129831, 1123031

Custodiol®: Nos. 1201111, 1129912, 1122911

7.4.3 Method of assigning patients to treatment groups

Patients who had signed informed consent and met all inclusion criteria and did not meet any exclusion criteria were randomized. Patients were assigned within the study centers to one of two treatment groups, Custodiol® or Custodiol-N, using a randomized block design. The randomization list was provided by the Coordination Centre for Clinical Trials (KKS) Heidelberg.

The randomization list was kept in safe and confidential custody at KKS and the sponsor's, who prepared the blinded medication and packaging.

7.4.4 Selection of doses in the study

See Section 7.4.1. Custodiol-N was administered in the same way as Custodiol®. The doses were according to the Summary of Product Characteristics (SmPC) of Custodiol®.

7.4.5 Selection and timing of dose for each patient

See Section 7.4.1.

7.4.6 Blinding

In addition to the study medication the investigator received a set of sealed envelopes, one for each randomization number. An identical set of sealed envelopes was held at the KKS Heidelberg. These envelopes contained information on the patient's study medication and were to be opened only under circumstances in which it was medically imperative to know what the patient was receiving. Date and reason for opening a sealed envelope was to be documented. If possible, the investigator conferred with the co-ordinating investigator before unblinding. The randomization envelopes were not to be opened by the investigator at the end of the study. All envelopes were to be collected by the monitor at the end of the study.

If it was medically imperative to know what study medication the patient was receiving, the investigator or authorized person should open the randomization envelope. The investigator or the person who broke the blind was to record the date and the reasons for doing so in the CRF, in the patient's medical record and on the randomization envelope. Whenever possible, the co-ordinating investigator and the sponsor should be contacted before the blind was broken.

The study medication was packed and labeled by the sponsor according to the randomization list. The study medication was packed and provided as 1 liter bottles or bags to the study centers.

The study medication was labeled according to § 5 of GCP-V.

7.4.7 Prior and concomitant therapy

Relevant additional treatments administered to the patients on entry to the study or at any time during the study, only inotropes, vasopressors, antiarrythmics, vasodilators and antihypertensives were regarded as concomitant treatments. Catecholamines (inotropes) were to be documented with actual dose and indication on the appropriate pages of the CRF during the 5 days of follow-up and each change of dose was to be documented separately. Other concomitant treatment was to be documented with start and end date and indication during the 5 days of follow-up.

7.4.8 Treatment compliance

Since the study medication was only applied by the investigators during surgery, evaluation of patient compliance was not applicable.

7.5 Efficacy and Safety Variables

7.5.1 Efficacy and safety measurements assessed and flow chart

The study consisted of 4 study periods: preoperative (baseline), intraoperative, postoperative (in the ICU) and follow-up. The primary hospitalization included part of the preoperative period, the intraoperative period, the postoperative ICU and the follow-up period.

Definitions of the study periods:

a) Preoperative (baseline) period: From a maximum of 2 weeks prior to surgery until immediately prior to start of anesthesia. The investigators had to bear in mind that any patient had to have ample time for being informed about the study, discussing the content of the patient information sheet, and signing the informed consent form. The preoperative period could include an ambulatory phase before admission to the institution. However, admission to the institution had to take place at least six hours prior to surgery.

b) Intraoperative period: From start of anesthesia to exit from the operating room.

c) Postoperative ICU period: From exit from the operating room to patient discharge from the ICU.

d) Follow-up period: From patient discharge from the ICU to up to 5 days after surgery and for mortality any time during post-op through Day 30.

A flow chart is provided in Table 7-2.

Table 7-2: Study flow chart

	Preoperative Baseline Period Max. 2 weeks **(# max. 7 days)** prior to surgery		Intraoperative Period (In the OR)					Postoperative Period (ICU)	5 (±1) days Follow-up	30 (±1) days Follow-up
		Arrival in the OR	Before Cardio-plegia	Cardioplegia Time		Before Cross Clamp Removal	Departure from OR			
				Cardiac Arresting*	Surgery					
Informed Consent Inclusion and Exclusion Criteria	X									
Medical History, NYHA	X								X	
Physical Examination	X								X	
Vital Signs (pulse rate, b.p.)	X#							X***	X***	
12-lead ECG	X#								X	
LV Function (Ejection Fraction)	X##									
Coronary Arteriography	X##									
Blood sampling for routine laboratory tests	X#							X 2 days post	X	
Blood sampling for TnT and CK-MB	X#							X**	X**	
Intraoperative events and procedures during induction and surgery and following cross clamp removal			X	X	X	X				
Catecholamine requirement (**cumulative dose)**			X	X	X	X	X	X	X	
Mortality any time during post-op through Day 30								X	X	X
Continuous ECG Monitoring				X****		X****				
Cardiac Index*****			X					X		

continued

continued

Collection of information on prior and concomitant medications	**Through day 5** →
Collection of information on adverse events	→
Collection of information on primary and/or secondary endpoints	→

* The time from the initiation of cardioplegic solution to cardiac arrest.

** Blood samples for TnT and CK-MB will be drawn at 4,8,12,16,20,24 hours ± 30 min and once a day on the days 2, 3, 4 and 5 after removal of aortic cross clamp.

*** Vital signs will be monitored 8, 16, 24 hours and once a day on the days 2, 3, 4 and 5 after removal of aortic cross clamp

max. 7 days prior to surgery.

Performance of coronary arteriography up to 90 days to the surgery is allowed, provided there have been no events that would suggest a change in the coronary anatomy.

**** Continuous cardiac monitoring is required during two intervals:
1) The time from the initiation of cardioplegic solution to cardiac arrest.
2) The time from cross clamp removal to the establishment of a stable rhythm.

***** Cardiac Index will be measured preoperatively, immediately upon the patient's entry to the ICU and 4,8,12,16,20,24 hours ± 30 min and once a day after removal of aortic cross clamp as long as the pulmonary catheter stays.

The primary endpoint was the creatine kinase muscle-brain (CK-MB) AUC within 24 hours (measurements 4, 8, 12, 16, 20, 24 hours ± 30 min) after release of the aortic cross clamp.

Secondary endpoints were:

- Catecholamine requirement on surgical intensive care unit (SICU) within 24 hours (cumulative dose)
- CK-MB (peak) on the days 2, 3, 4 and 5 after removal of aortic cross clamp
- Cardiac troponin T 4,8,12,16,20,24 hours ± 30 min and on the days 2, 3, 4 and 5 after release of the aortic cross clamp
- Cardiac index
- Defibrillation
- Requirement for intraaortic balloon pump (IABP)
- Blood pressure
- Length of SICU stay
- Duration of mechanical ventilation (intubation to extubation)
- Pulmonary and systemic vascular resistance
- Occurrence, severity, type, and duration of cardiac arrhythmias[1]
- Laboratory parameters
- Repeated patient transfer to SICU
- Mortality any time during post-op through Day 30
- Safety (documentation and reporting of adverse events [AEs] and serious adverse events [SAEs]

Patients had their **cardiac index** measured immediately after placement of the Swan-Ganz catheter and thereafter every 4 hours for 24 hours ± 30 min after removal of the aortic clamp or until the patient was discharged from the intensive care unit (ICU) (whichever occurred first). Hemodynamics were assessed using the thermodilution technique in which 5 mL of

[1] Please note that the analysis of cardiac arrhythmias was finally restricted to the evaluation of type and incidence.

cold saline solution is injected into the proximal port of the catheter and its temperature recorded at the tip positioned in the main trunk of the pulmonary artery. Preoperative measurements in each patient served as baseline comparisons versus post-cardioplegic arrest values to determine if there was impaired myocardial function. The cardiac index was recorded, based upon correction for body surface area (BSA). The following formula was applied:

Cardiac index = CO/BSA

CO = Cardiac output (L/min) determined by thermodilution method; BSA = Body surface area

For further information on the parameters documented at particular points in time (i.e. at baseline, during the intraoperative period, during the postoperative period and during the follow-up period) please refer to Sections 7.1, 7.2, 7.3 and 7.5 of the study protocol).

For definitions of AE, SAE, unexpected AE and suspected unexpected serious adverse reaction (SUSAR) see Sections 9.1.1, 9.1.2, 9.1.3 and 9.1.4 of the study protocol.

Due to the study design and the patient population, the following AEs were expected to occur:

- Surgical complications (specific to the performed operation)
- General postoperative complications
- Re-interventions for complications
- Cardiac death
- Myocardial infarction
- Unstable angina
- Heart failure
- Life-threatening arrhythmias

Despite their expectedness the occurrences listed above were to be properly recorded like all other AEs.

AEs were to be documented from the time of study inclusion up to the follow-up visit on Day 5 (± 1 day). All patients who presented with AEs, whether considered associated with the use of the study medication or not, were to be monitored by the responsible investigator to determine their outcome. The clinical course of the AE was to be followed up until

resolution/normalization of changed (laboratory) parameter or until it had changed to a stable condition. AEs were to be classified with respect to their intensity (mild, moderate, severe), relatedness to the study medication (related, probable, possible, unlikely, not related, not assessable), outcome (recovered/resolved, recovering/resolving, not recovered/not resolved, recovered/resolved with sequelae, fatal, unknown), action taken with respect to study medication (dose not changed, dose reduced, dose increased, drug withdrawn, unknown, not applicable) and countermeasures (no action taken, drug treatment, others). For further information refer to Section 9.2 of study protocol. For reporting requirements with respect to SAEs refer to Section 9.3 of the study protocol.

7.5.2 Appropriateness of measurements

The cardiac enzyme CK-MB and the more sensitive cardiac troponin T are standard markers for myocardial cell injury and have been frequently used in clinical studies evaluating cardio-protective measures during cardiac surgery (29,30).

7.5.3 Drug concentration measurements

Not applicable.

7.6 Data Quality Assurance

7.6.1 Data management

All protocol-required information collected during the study was entered by the investigator, or a designated representative, in the electronic case report form (eCRF). Patient data was documented pseudonymously. The investigator, or a designated representative, should complete the eCRF pages as soon as possible after the information was collected, preferably on the same day when a study patient was seen for an examination, treatment, or any other study procedure. Any pending entries were to be completed immediately after the final examination. Explanation should be given for all missing data.

The cooperation unit KKS Heidelberg checked completeness, validity and plausibility of data by validating programs, which generated queries. In case of implausibilities, "warnings" were produced. A responsible investigator was obliged to either correct the implausible data or to confirm its authenticity and to give appropriate explanation. If not corrected, the data was flagged, rendering a check of all questionable entries conveniently possible. A responsible

monitor checked all flagged data and generated questions that were sent back to the responsible investigator. The investigator was to resolve all discrepancies.

Further checks for plausibility, consistency, and completeness of the data were performed after completion of the study. Queries were generated on the basis of these checks combined with a visual control by a responsible data manager.

Each CRF section was signed by the investigator or by his or her authorized representative.

Data management was accomplished according to the appropriate standard operating procedures (SOPs) valid in the KKS.

7.6.2 Monitoring

The study was performed in accordance with Good Clinical Practice and thus required regular monitoring visits. Monitoring was done by personal visits from a clinical monitor according to the SOPs of the KKS. Monitoring visits were performed based on patient accruals and availability of CRFs. The monitor reviewed the entries into the CRFs on the basis of source documents. Details of monitoring (i.e. frequency of visits and/or extent of source data verification [SDV]) were specified in the monitoring manual for this study. Between these visits, contacts with study center personnel were made by telephone, by fax or by mail to ensure that the study was conducted according to the protocol and the regulatory requirements.

Prior to the monitor's visit, the investigator was to make sure that all data were recorded in the CRFs. The investigator was to allow the monitor access to the source data and essential documents and was to provide support at all times to the monitor.

During the monitoring visit the monitor checked, with the investigator, the progress of the study and protocol compliance as assessed by the data recorded in the CRFs. The investigator(s) agreed to permit the monitor to be present to observe the study procedure in 1 or more patients.

7.7 Statistical Methods and Determination of Sample Size

7.7.1 General considerations

SAS Version 9.2 was used for all descriptive and analytic methods.

7.7.2 Analysis populations

The full analysis set (FAS) comprised all patients randomized into a treatment group and receiving cardioplegic solution. The per-protocol (PP) set comprised all patients in the FAS who met the eligibility criteria, who received the cardioplegic solution as randomized, and who had (for the determination of the AUC) blood samples at times not varying by more than 30 minutes from the scheduled time. Occasional missing samples were interpolated and did not lead to exclusion from the PP set.

The safety set comprised all patients who received one of the two treatments, regardless of protocol compliance.

The primary hypothesis was tested using the FAS as well as the PP set.

7.7.3 Demography and baseline characteristics

Age was tabulated by sex against treatment group using minimum, median, maximum, mean and standard deviation. Body height in cm and body weight in kg and the derived body mass index were tabulated against treatment group using minimum, median, maximum, mean and standard deviation.

The severity of the underlying disease was tabulated using the New York Heart Association (NYHA) scale by category against treatment group using absolute and relative frequency.

Findings in electrocardiographic and physical examinations were coded using the Medical Dictionary for Regulatory Activities (MedDRA) in English language Version 15.0 and tabulated using absolute frequency for every finding.

Findings in coronary arteriography were tabulated using absolute and relative frequencies.

Medical history terms were coded using MedDRA Version 15.0 and tabulated against treatment group.

The left ventricular ejection fraction was tabulated against treatment group using minimum, median, maximum, mean and standard deviation.

Laboratory measurements at screening were tabulated against treatment group using minimum, median, maximum, mean and standard deviation, as were systolic and diastolic blood pressure and heart rate.

7.7.4 Analysis of primary endpoint

7.7.4.1 Model assumptions

The AUC from 4 to 24 hours for CK-MB was derived from the single measurements using the trapezoidal method on the scheduled time points. For modeling purposes the AUC was transformed by natural logarithm, as the parameter is known to have a right-skewed distribution and for a more natural approach to relative differences.

7.7.4.2 Null Hypotheses

As both, the FAS and the PP set were taken to answer the study question, there were essentially two null hypotheses, named H_{0F} and H_{0P}, here:

H_{0F}: The AUC for CK-MB in the Custodiol-N treatment group exceeds, for the FAS, the AUC for CK-MB in the Custodiol® treatment group by at least 30 per cent.

H_{0P}: The AUC for CK-MB in the Custodiol-N treatment group exceeds, for the PP set, the AUC for CK-MB in the Custodiol® treatment group by at least 30 per cent.

7.7.4.3 Test and test statistic

The test statistic (Z_F for the FAS and Z_P for the PP set) was the difference of the means of logarithms of the AUC values between the Custodiol-N and Custodiol® treatment groups, added to the logarithm of 130%, and divided by the common standard error of the logarithm of the AUC.

7.7.5 Analysis of secondary endpoints

The $AUC_{4\text{-}24h}$ for troponin T was determined in the same manner as for the primary endpoint: The log AUC (obtained by the trapezoidal rule) was averaged in either treatment group; the difference of the means was divided in order to obtain a t-statistic to calculate a 95% confidence interval (CI) for the relative difference between treatments.

The demand for catecholamines was categorized into "not at all", "yes, up to 6 hours after opening the aortic cross-clamp", "yes, more than 6 hours after opening the aortic cross-clamp". This binned variable was tabulated against treatment group using absolute and relative frequencies.

The maximum values for CK-MB and troponin T over the first 5 days was elicited and tabulated against treatment group using the number of nonmissing and missing values, minimum, median, maximum, mean and standard deviation.

Cardiac index, obtained at the same post-surgical time points as the blood samples for CK-MB and troponin T, was tabulated by time point against treatment group using the number of nonmissing and missing values, minimum, median, maximum, mean and standard deviation.

Usage of a defibrillator, readmission to ICU within 5 days and usage of an intraaortic balloon pump (the latter not actually applicable) (all yes/no questions) were tabulated against treatment group using absolute and relative frequencies.

Systolic and diastolic blood pressure up to 5 days after re-opening of the aortic cross-clamp were tabulated against treatment group using the number of nonmissing and missing values, minimum, median, maximum, mean and standard deviation.

As a sensitivity analysis for the primary hypothesis, the log AUC for 4-24 hour CK-MB was used as a response variable in a linear model with study center and treatment group as explanatory variables. A subgroup analysis for the study center Heidelberg was performed as most patients were enrolled in Heidelberg.

7.7.6 Safety analysis

AEs were coded according to MedDRA and listed by treatment group and Preferred Term using the variables patient number, AE number, first and last study day, relatedness, and outcome. All events were tabulated against treatment group using absolute frequencies and were tabulated by MedDRA System Organ Class against treatment group, where number of single events and number of patients with events were both tabulated. Also, all AEs as well as SAEs were listed by treatment group, patient and MedDRA Preferred Term.

Laboratory parameters were tabulated by visit/time point against treatment group using the number of nonmissing and missing values, minimum, median, maximum, mean and standard deviation. Shift tables were generated for cross-tabulation of deviation from reference ranges at baseline versus at follow-up. Vital signs were also tabulated by visit against treatment group in the same fashion as laboratory parameters.

7.7.7 Interim analysis

After the analysis of 50 patients, an estimation of the sample size necessary to answer the study hypothesis was planned. If either one of Z_F and Z_P was below the critical value of

$u_{1-\alpha_1} \approx -0.5244$ (where u was the quantile of the standard normal distribution and α_1 was set to the futility threshold of 70%), the study would have had to be stopped for futility. The sample size necessary for the completion of the study was to be calculated as $\frac{\sqrt{n_1}Z_1 + \sqrt{n_2}Z_2}{\sqrt{n_1 + n_2}} < u_{1-\alpha_2/2}$ with the side condition $\sqrt{n_2} > \frac{\sigma(Z_2 + u_{1-\beta_2})}{\delta}$. The necessary sample size for the rest of the study was to be calculated separately for the FAS and the PP set, and the maximum of these two values was to be adopted for the remaining number of patients necessary to complete the study.

For results of the interim analysis see Section 9.4.2.3

7.7.8 Determination of sample size

The sample size for the study was determined in order to yield approximately 90% power for establishing clinical equivalence between treatments with respect to the primary endpoint – CK-MB. The non-inferiority margin was set to a CK-MB level of 130 percent (AUC in the first 24 hours after removal of aortic cross clamp) of the Custodiol-N group compared with the Custodiol® group. The inferiority margin was selected in order to indirectly derive an advantage of Custodiol-N over not using any cardioplegic solution. In a randomized study on 103 patients (28), HTK solution was compared with aortic cross-clamping with respect to peak values of CK-MB, rendering levels of 180 percent of the control group compared with the HTK solution group. A non-inferiority margin of 130 percent therefore ruled out that Custodiol-N, despite rejection of the null hypothesis, was worse than using no solution at all.

To consider the case the null hypothesis of non-inferiority being true, accrual took place in 3 stages. In the first stage, 10 patients were administered Custodiol-N and evaluated for safety. These patients were not included in the final analysis of efficacy. In the second stage, 50 patients were enrolled. After the second stage, a comparison for efficacy was performed. The study was to stop for futility if the hypothesis of non-inferiority could not be rejected at the α_1=0.7 level. Otherwise, the study was to continue with at least 50 more patients as less than 100 patients were considered inadequate for a pivotal study. The patient number in the third stage was to be calculated using weighted inverse normalized p-values as described in Lehmacher and Wassmer (1999) (31). For further details see Section 7.7.7. Patient recruitment continued during the data clearing and analysis process after the inclusion of the first 50 patients. Unless the study had to be stopped for futility, at least 50 more patients were to be recruited. If the number of treatment failures among the first 50 patients was so

high that an early termination for futility became a possibility, the DSMB could recommend the interruption of recruitment.

7.8 Changes in the Conduct of the Study or Planned Analyses

7.8.1 Protocol amendments

There was 1 amendment to the original protocol (dated 31 Jan 2011).

In Amendment 1, issued on 20 October 2011, after enrollment of 17 patients, the following modifications were introduced:

- Two additional study centers were introduced.
- In Section "Sample size and non-inferiority margin justification" it was added that patient recruitment would continue during the data clearing and analysis process after the inclusion of the first 50 patients. Unless the study had to be stopped for futility, at least 50 more patients would have to be recruited. If the number of treatment failures among the first 50 patients was so high that an early termination for futility became a possibility, the DSMB could recommend the interruption of recruitment.
- In Section "Intraoperative event recording" the required documentation with respect to bypass grafts was specified more precisely.
- In Section "Clinical laboratory tests" the evaluation of interleukin-6 was dropped.
- In Section "Expedited reporting" changes in personnel were introduced.
- In Section "Analysis methods" it was added that in the sample size calculation for the third stage, the number of patients "n_2" was rounded up to 50 if it was lower than 50 in order to ensure a minimum number of 100 patients in the study.
- Minor editorial changes were made.

7.8.2 Other changes in study conduct

There were no other changes in study conduct.

7.8.3 Changes in planned analysis

During the course of the trial it was found out that at 1 study center (Jena), the CK-MB values were determined by concentration and not by enzymatic reaction. There is no established

method of conversion between U/L and mol/L, so all values for CK-MB were effectively missing from that study center. In order not to lose data from these patients, Multiple Imputation methods were used. By estimating the CK-MB values using a regression model with the concurrently determined troponin T value as the only explanatory variable, the data from these patients could be used in a meaningful way.

In addition, the statistical analysis of the CKMB peak values was also performed without the extrapolated data, only including directly measured CKMB values by enzymatic reaction.

The procedures "Re-admission to ICU" and "Defibrillation" up to 5 days after surgery were only reported for 1 patient each. Therefore, no odds ratios or other comparative measures were generated as they would not add to the interpretation of these results.

For the secondary endpoints "duration of mechanical ventilation" and "pulmonary vascular resistance", two-sided Wilcoxon rank sum tests for difference have been calculated. For testing the difference with respect to catecholamine intake mode, Mantel-Haenszel’s Chi-squared test for ordinal values has been used.

8 STUDY PATIENTS

8.1 Disposition of Patients

An overview on patient disposition is provided in Table 8-1. A total of 112 patients entered the study. This included the "Custodiol-N only treated cohort" of 10 patients who were treated with Custodiol-N and evaluated for safety in the first stage of the study. A total of 50 patients were randomized to Custodiol-N and 52 patients to Custodiol®. In all but 1 patient in the Custodiol-N group who finally received an intervention without the use of cardioplegia, study medication was applied during the operation. The majority of randomized patients (97.1%) completed the 30-day follow-up. One patient in each group died during the study (including the 30-day follow-up period) (cf. post-text Table 14.2.12). One further patient in the Custodiol® group died on Day 43 (cf. post-text Tables 14.1.1, 14.2.10 and 14.3.2.1, and post-text Listing 16.2.1.1).

Table 8-1: Patient disposition (all patients)

	Custodiol-N	Custodiol®	Total
Patients who			
entered the study	*60*	*52*	*112*
were not randomised ("Custodiol-N only treated cohort" for safety evaluation in the first stage of the study)	*10*	*0*	*10*
were randomised – N (%)	50 (100.0)	52 (100.0)	102 (100.0)
were treated – n (%)[a]	49[b] (98.0)	52 (100.0)	101[c] (99.0)
completed the study (period of treatment) – n (%)[a]	49 (98.0)	52 (100.0)	101 (99.0)
completed 30-day follow-up – n (%)[a]	48 (96.0)	51 (98.1)	99 (97.1)
Cause of discontinuation (including 30-day follow-up)			
Death[d]	1	1	2

[a] = Percentages calculated by the author on the basis of randomized patients.
[b] = The total number of patients treated with Custodiol-N (including the "Custodiol-N only treated cohort") was 59.
[c] = The total number of patients treated (including the "Custodiol-N only treated cohort") was 111.
[d] = One further patient in the Custodiol® group died on Day 43.
Note: One patient (patient no. 41) who was randomized into the Custodiol-N group subsequently received an intervention without the use of cardioplegia.
Data source: Post-text Tables 14.1.1, 14.2.10 and 14.3.2.1, and post-text Listing 16.2.1.1.

8.2 Protocol Deviations

One patient (patient no. 41) had been randomized into a treatment group (Custodiol-N) before the surgical team opted for an intervention not using cardioplegia. The cardioplegic solution was left unopened but had already been taken out of the cooling system so it could not be reused. The blind was maintained during this procedure. This patient was excluded from all analysis populations (post-text Listing 16.2.1.1).

If patients had their blood sampled at time points differing from the scheduled time point by more than 30 minutes, or if they had been randomized despite violating an eligibility criterion, they were excluded from the per protocol set.

No violations of eligibility criteria were identified.

Six patients in the Custodiol-N group (patients nos. 11, 35, 1022, 1023, 1082 and 1090) and 8 patients in the Custodiol® group (patients nos. 12, 32, 33, 92, 1011, 1024, 1081 and 1084) had blood sampling time points deviating by more than 30 minutes from the scheduled time points. These patients were excluded from the PP set (post-text Listing 16.2.2.1).

9 EFFICACY EVALUATION

9.1 Data Sets Analyzed

All patients in whom study treatment was applied were included into the safety set. The number of patients in the safety set was slightly higher in the Custodiol-N group since it also contained the non-randomized "Custodiol-N only treated cohort" of 10 patients who were treated with Custodiol-N and evaluated for safety in the first stage of the study. With the exception of the "Custodiol-N only treated cohort" all patients from the safety set were also included into the FAS. Six patients in the Custodiol-N group and 8 patients in the Custodiol® group had blood sampling time points deviating by more than 30 minutes from the scheduled time points and were therefore excluded from the PP set. Apart from for the slightly higher number of patients in the Custodiol-N safety set, the sample sizes in the analysis sets were comparable in the treatment groups (cf. post-text Table 14.1.1 and post-text Listing 16.2.2.1).

The study was conducted in 4 study centers. It should be noted that the vast majority of patients was included in the study center in Heidelberg. Of the 101 patients in the FAS, 82 (41 patients in each treatment group) were included in Heidelberg (cf. post-text Table 14.2.3).

Table 9-1: Data sets analyzed

	Custodiol-N	Custodiol®	Total
Safety set	59	52	111
Full analysis set (FAS)	49	52	101
Per-protocol (PP) set	43	44	87

Data source: Post-text Table 14.1.1

9.2 Demographic and Other Baseline Characteristics

9.2.1 Demographic and baseline disease characteristic

Demography and baseline characteristics which were overall comparable between the treatment groups are summarized in Table 9-2 for the FAS. The vast majority of patients (approximately 80%) were men. The mean age was 68.1 ± 8.0 years in the Custodiol-N group and 66.4 ± 8.5 years in the Custodiol® group (cf. post-text Table 14.1.2). In both treatment groups approximately half of the patients presented with NYHA class II (cf. post-text Table 14.1.8).

Summary statistics of laboratory parameters at screening which did not point to relevant differences between the treatment groups are provided in post-text Table 14.1.5 (hematology

and serum chemistry) and in post-text Table 14.1.6 and 14.1.7 (urinalysis). Interestingly, elevated baseline values for troponin T and/or CK-MB were observed in at least 1 patient in both treatment groups (see range with maximum value for both parameters in Table 9-2).

Table 9-2: Demography and baseline characteristics (full analysis set)

		Custodiol-N (N=49)	Custodiol® (N=52)
Sex	Male, n (%)[a]	39 (79.6)	42 (80.8)
	Female, n (%)[a]	10 (20.4)	10 (19.2)
Age [years]	Mean ± Std	68.1 ± 8.0	66.4 ± 8.5
	Range	44–80	45–80
Body weight [kg]	Mean ± Std	82.3 ± 15.0	85.6 ± 18.1
	Range	48–140	51–132
Body Mass Index [kg/m^2]	Mean ± Std	28.2 ± 4.0	28.5 ± 4.9
	Range	19–42	20–46
Systolic blood pressure [mmHg]	Mean ± Std	139 ± 20	138 ± 22
	Range	100–190	83–200
Diastolic blood pressure [mmHg]	Mean ± Std	76 ± 13	77 ± 11
	Range	40–110	51–110
Heart rate [bpm]	Mean ± Std	74 ± 12	73 ± 13
	Range	51–100	49–110
Left ventricular ejection fraction [%]	Mean ± Std	62.5 ± 8.1	59.3 ± 10.5
	Range	40–80	39–85
NYHA class	I, n (%)	7 (14.3)	7 (13.5)
	II, n (%)	24 (49.0)	27 (51.9)
	III, n (%)	17 (34.7)	12 (23.1)
	IV, n (%)	1 (2.0)	6 (11.5)
Coronary arteriography findings	2-vessel disease, n (%)	6 (12.2)	5 (9.6)
	3-vessel disease, n (%)	43 (87.8)	47 (90.4)
Troponin T [pg/mL]	N	39	42
	Mean ± Std	28.7 ± 55.1	19.5 ± 36.4
	Range	3.0–317.0	3.0–188.0
CK-MB [U/L]	N	35	42
	Mean ± Std	20.8 ± 9.6	19.0 ± 12.2
	Range	7.2–45.0	1.0–82.0

[a] = Percentages calculated by the author.
Abbreviations: bpm=beats per minute; NYHA=New York Heart Association
Data source: Post-text Tables 14.1.2, 14.1.3, 14.1.5, 14.1.8 and 14.1.11

9.2.2 Past and concomitant diseases

Most frequently reported in the medical history were cardiac disorders (total: 93.1%; Custodiol-N: 93.9%; Custodiol®: 92.3%), vascular disorders (total: 89.1%; Custodiol-N: 87.8%; Custodiol®: 90.4%) and metabolism and nutrition disorders (total: 78.2%; Custodiol-N: 81.6%; Custodiol®: 75.0%) (cf. post-text Table 14.1.10) (FAS).

Most frequently reported conditions (Preferred Terms) were coronary artery disease (total: 88.1%; Custodiol-N: 89.8%; Custodiol®: 86.5%), hypertension (total: 84.2%; Custodiol-N:

81.6%; Custodiol®: 86.5%) and hyperlipidemia (total: 51.5%; Custodiol-N: 51.0%; Custodiol®: 51.9%) (cf. post-text Table 14.1.9) (FAS).

A difference between the treatment groups was observed with respect to psychiatric disorders which were more common in the Custodiol-N group (26.5%) than in the Custodiol® group (13.5%). This almost exclusively referred to tobacco abuse reported in 24.5% of patients in the Custodiol-N group and in 13.5% of patients in the Custodiol® group. Further conditions with ≥ 10% difference in prevalence between the treatment groups were angina pectoris (Custodiol-N: 20.4%; Custodiol®: 3.8%) and hypercholesterolemia (Custodiol-N: 18.4%; Custodiol®: 7.7%)

9.2.3 Prior and concomitant medication

A by-patient listing of concomitant medication is provided in post-text Listing 16.2.9.

9.3 Measurements of Treatment Compliance

Not applicable since the study medication was only applied by the investigators during surgery.

9.4 Efficacy Results and Tabulations of Individual Patient Data

9.4.1 Analysis of efficacy

9.4.1.1 Primary endpoint

The primary endpoint was the CK-MB AUC within 24 hours (measurements 4, 8, 12, 16, 20, 24 hours ± 30 min) after release of the aortic cross clamp (CK-MB $AUC_{4\text{-}24h}$).

Results with respect to the comparison of the treatment groups are summarized in Table 9-3. [*Please note that the percentage difference denotes the antilog of the differences of means of log(AUC), namely of the mean log(AUC) in Custodiol-N patients, deducted from the mean log(AUC) in Custodiol patients. A value above zero hints to a higher level in patients receiving standard treatment. The lower and upper confidence limits have been calculated at the local 95% level on the log(AUC) scale and transformed by the antilog (exponential function). The two rightmost columns denote p-values for the null hypothesis of inferiority of Custodiol-N by 30% and for the null hypothesis of inferiority of Custodiol-N by 0%. Both p-values refer to one-sided hypotheses.]*

The primary analysis statistically confirmed non-inferiority of Custodiol-N compared with Custodiol® as determined by the CK-MB AUC_{4-24h} (p< 0.0001 at the 30% non-inferiority margin both in the FAS and in the PP set; cf. post-text Table 14.2.2).

The mean CK-MB AUC_{4-24h} was descriptively lower in the Custodiol-N group (779 ± 439 [median: 620; range: 270-2672] h*U/L) than in the Custodiol® group (878 ± 549 [median: 742; range: 100-2550] h*U/L; cf. post-text Table 14.2.3).

Table 9-3: Primary analysis: Treatment effect on CK-MB AUC_{4-24h}

Parameter	**% Difference** (Custodiol® minus Custodiol-N)[a]	**[95% CI]**	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 30%)	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 0%)	**Analysis set**
CK-MB	2.7	[-8.3; 15.0]	<0.0001	0.3192	FAS
	2.5	[-9.2; 15.8]	<0.0001	0.3427	PP set

[a] = Antilog of difference of mean log(AUC) in Custodiol® patients minus mean log(AUC) in Custodiol-N patients.
Abbreviations: CI = Confidence interval; FAS = Full analysis set; H_0 = Null hypothesis; PP = Per-protocol
Data source: Post-text Table 14.2.2

9.4.1.1.1 Sensitivity analysis

Refer to Section 9.4.2.4.

9.4.1.2 Secondary endpoints

9.4.1.2.1 Treatment effect on troponin T AUC_{4-24h}

As summarized in Table 9-4, results with respect to the troponin T AUC_{4-24h} supported the result of non-inferiority of Custodiol-N compared to Custodiol®, obtained in the primary analysis (p=0.0027 in the FAS and p=0.0022 in the PP set [30% non-inferiority margin]; cf. post-text Table 14.2.2).

Summary statistics for troponin T AUC_{4-24h} showed descriptively higher mean values in the Custodiol-N group (13499 ± 6513 [median: 12457; range: 2922-28020] h*pg/mL) than in the Custodiol® group (12991 ± 8348 [median: 11992; range: 1640-46640] h*pg/mL; cf. post-text Table 14.2.3).

Table 9-4: Secondary analysis: Treatment effect on troponin T $AUC_{4\text{-}24h}$

Parameter	**% Difference** (Custodiol® minus Custodiol-N)[a]	**[95% CI]**	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 30%)	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 0%)	**Analysis set**
Troponin T	-9.2	[-19.1; 1.9]	0.0027	0.9495	FAS
	-7.7	[-18.5; 4.6]	0.0022	0.8966	PP set

Note: Secondary analysis → P-values have to be interpreted descriptively.
[a] = Antilog of difference of mean log(AUC) in Custodiol® patients minus mean log(AUC) in Custodiol-N patients.
Abbreviations: CI = Confidence interval; FAS = Full analysis set; H_0 = Null hypothesis, PP = Per protocol
Data source: Post-text Table 14.2.2

9.4.1.2.2 Treatment effect on CK-MB and troponin T peak values

CK-MB peak values were descriptively slightly lower under Custodiol-N with 49 ± 30 U/L (median: 38 U/L; range 18-190 U/L) in the Custodiol-N group and 58 ± 40 U/L (median: 45 U/L; range 13-210 U/L) in the Custodiol® group. Troponin P peak values were comparable in both groups with 946 ± 471 pg/mL (median: 904 pg/mL; range 191-2404 pg/mL) in the Custodiol-N group and 931 ± 554 pg/mL (median: 848 pg/mL; range 160-3507 pg/mL) in the Custodiol® group (cf. post-text Table 14.2.3).

As summarized in Table 9-5, comparison of the treatment groups with respect to CK-MB and troponin T peak values supported the result of non-inferiority of Custodiol-N compared to Custodiol®, obtained in the primary analysis (p<0.0001 for both parameters both in the FAS and in the PP set). For CK-MB, evaluation at the 0% margin (p=0.032 in the FAS; p=0.0782 in the PP set) showed more favorable results under Custodiol-N. This was even more pronounced in the complete case analysis where the extrapolated data from the study center in Jena were omitted (p=0.0239) (cf. post-text Table 14.2.4).

Table 9-5: Secondary analysis: Treatment effect on CK-MB and troponin T peak values

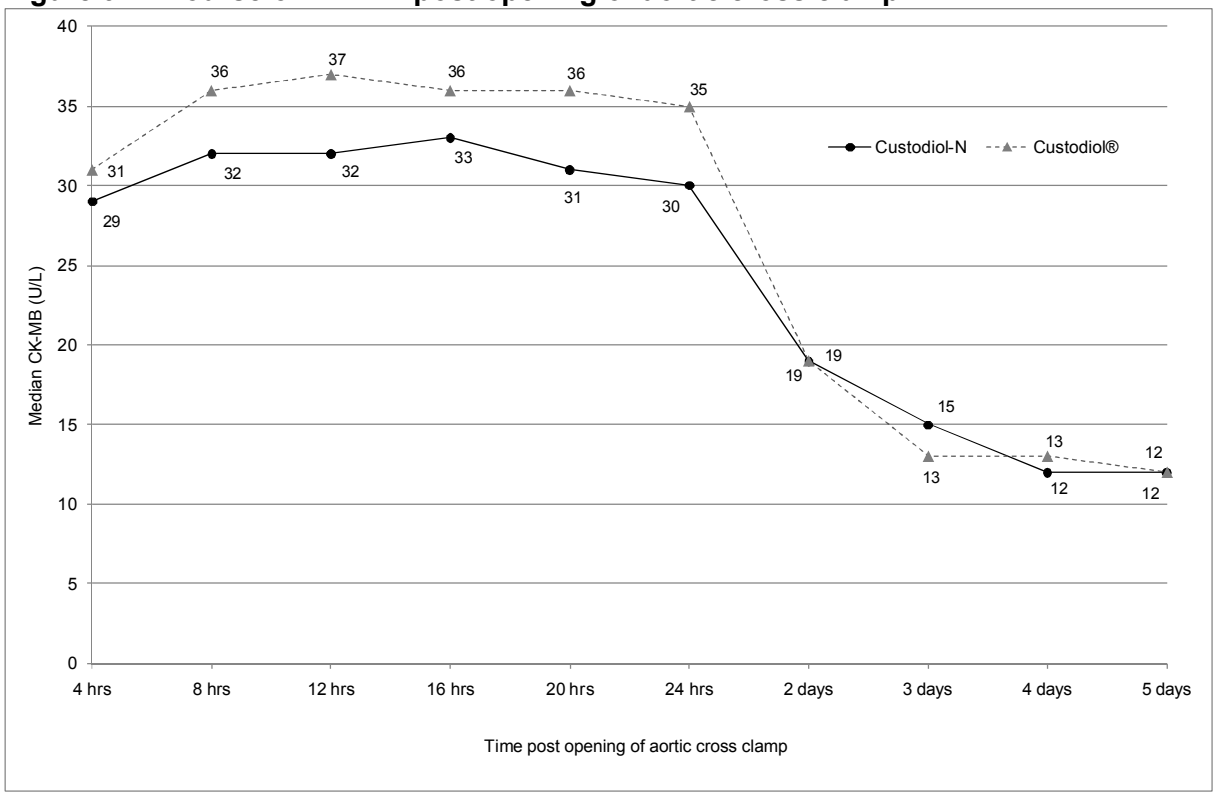
Parameter	**% Difference** (Custodiol® minus Custodiol-N)	**[95% CI]**	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 30%)	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 0%)	**Analysis set**
CKMB	10.9	[-0.6; 23.9]	<0.0001	0.0322	FAS
	9.2	[-3.4; 23.3]	<0.0001	0.0782	PP set
	12.4	[0.1; 26.2]	<0.0001	0.0239	Jena excl.[a]
Troponin T	-3.0	[-13.0; 8.2]	<0.0001	0.7075	FAS
	-0.2	[-11.4; 12.3]	<0.0001	0.5143	PP set

Note: Secondary analysis → P-values have to be interpreted descriptively.
[a]: Complete case analysis with no data extrapolation.
Abbreviations: CI = Confidence interval; FAS = Full analysis set; H_0 = Null hypothesis; PP = Per-protocol
Data source: Post-text Table 14.2.4

9.4.1.2.3 Course of CK-MB

As shown in Figure 9-1, CK-MB values post opening of the aortic cross clamp showed a similar course in both treatment groups with increased median values at 4 hours and a further slight increase at 8 hours followed by a clear decrease at Day 2. During the first 24 hours, median values in the Custodiol-N group were slightly lower than in the Custodiol® group (cf. post-text Table 14.2.1).

Figure 9-1: Course of CK-MB post opening of aortic cross clamp

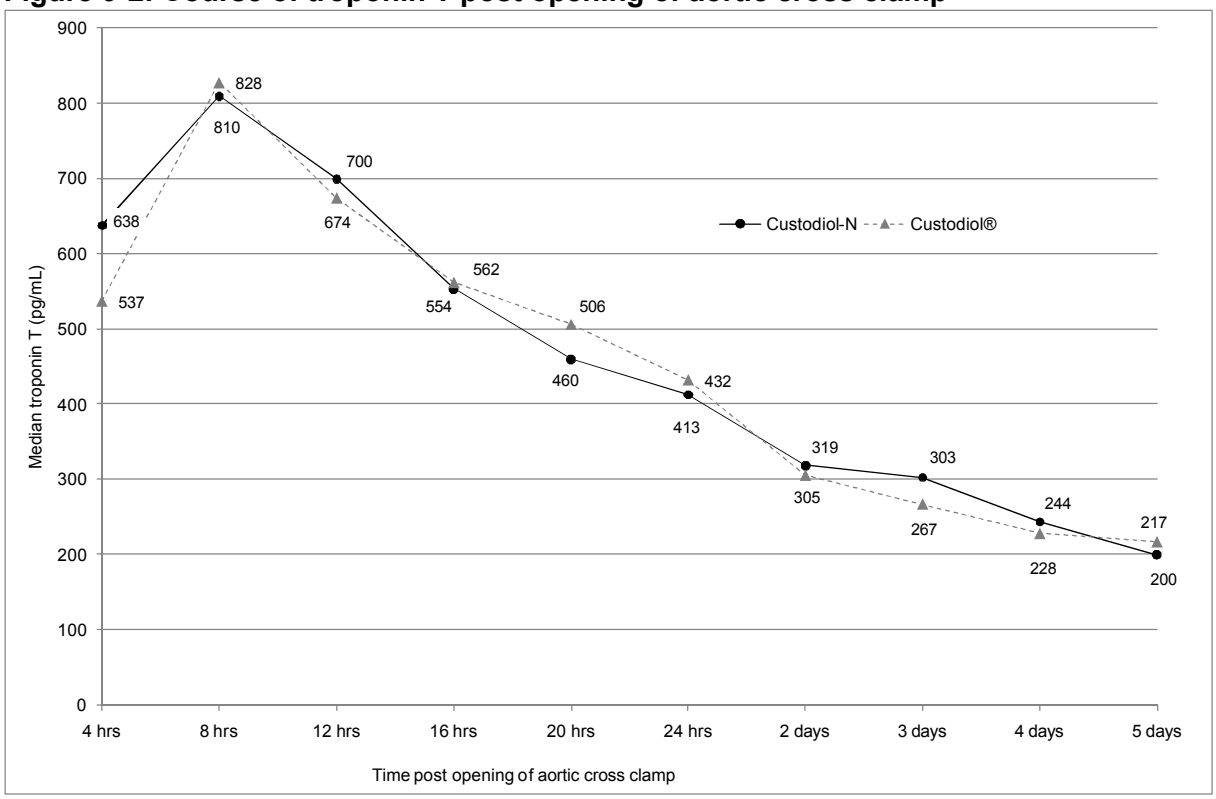


Please note non-linear scaling on x-axis. Abbreviations: hrs = hours
Source: Post-text Table 14.2.1, full analysis set

9.4.1.2.4 Course of troponin T

As shown in Figure 9-2, troponin T values post opening of the aortic cross clamp showed a similar course in both treatment groups with increased median values at 4 hours and a further increase at 8 hours followed by a gradual and constant decrease. At 4 hours, median values in the Custodiol-N group were higher than in the Custodiol® group (cf. post-text Table 14.2.1).

Figure 9-2: Course of troponin T post opening of aortic cross clamp



Please note non-linear scaling on x-axis. Based on non-missing values. Abbreviations: hrs = hours
Source: Post-text Table 14.2.1, full analysis set

9.4.1.2.5 Catecholamine requirement on surgical intensive care unit (SICU) within 24 hours

The total doses of catecholamines administered during 24 hours post opening of the aortic cross clamp are shown in Table 9-6.

Table 9-6: Total dose of catecholamines up to 24 hours post opening of aortic cross clamp (full analysis set)

		Custodiol-N	Custodiol®
Adrenaline, µg/kg	N	49	52
	Mean ± SD	1.6 ± 10.7	0.6 ± 3.0
	Range	0.0 – 75.0	0.0 – 21.5
Dobutamine, µg/kg	N	49	52
	Mean ± SD	25.7 ± 72.2	30.4 ± 71.1
	Range	0.0 – 397.5	0.0 – 302.5
Noradrenaline, µg/kg	N	49	52
	Mean ± SD	13.4 ± 23.9	7.4 ± 9.4
	Range	0.0 – 131.0	0.0 – 43.4

Data source: Post-text Table 14.2.5

An evaluation of the last administration of catecholamines is shown in Table 9-7. There were no relevant differences between the treatment groups (p=0.12; Mantel-Haenszel Chi-Square test; cf. Section 16.1.9) (cf. post-text Table 14.2.5).

Table 9-7: Last administration of catecholamines (full analysis set)

	Custodiol-N N=49 n (%)	Custodiol® N=52 n (%)	Total N=101 n (%)
No administration of catecholamines	3 (6.1)	7 (13.5)	10 (9.9)
Last admin. < 6 hrs post opening of aortic cross clamp	5 (10.2)	8 (15.4)	13 (12.9)
≥ 6 hrs post opening of aortic cross clamp	41 (83.7)	37 (71.2)	78 (77.2)

Data source: Post-text Table 14.2.5

9.4.1.2.6 Cardiac index

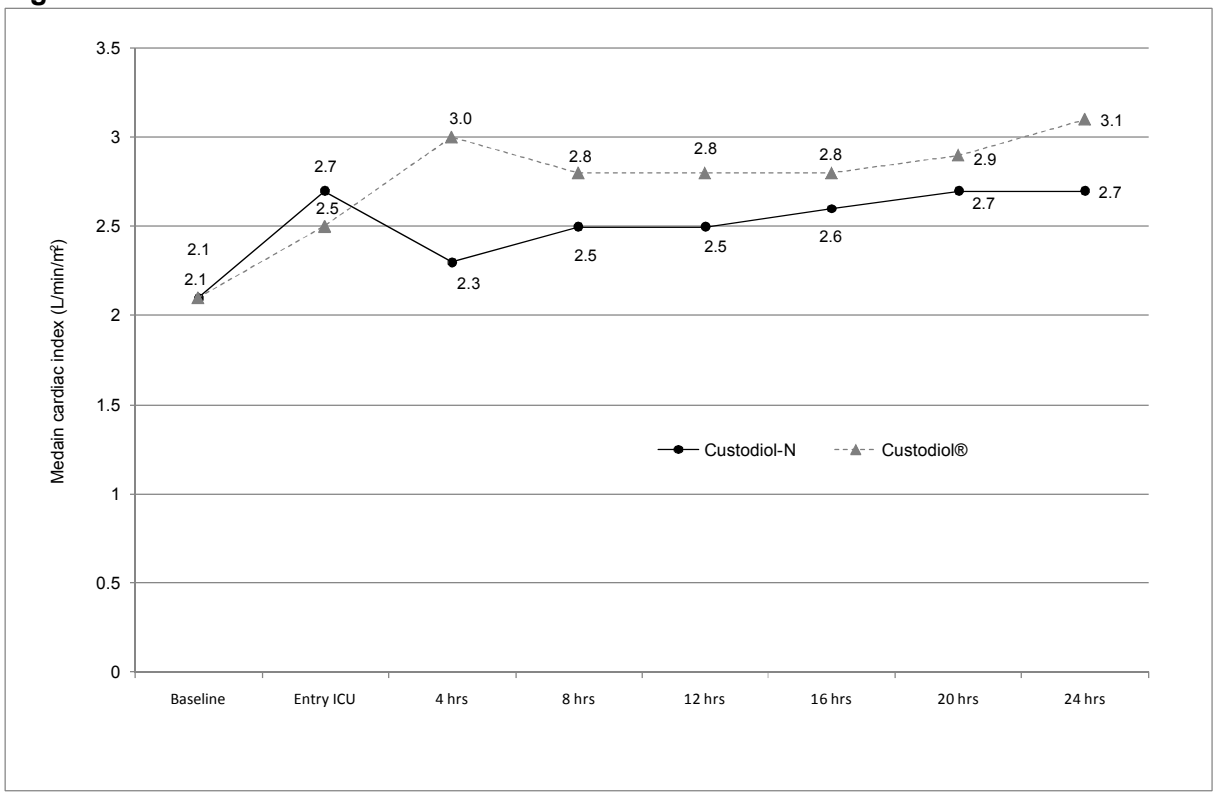
As shown in Table 9-8 and Figure 9-3, the mean/median cardiac index which was similar in both treatment groups at baseline (placement of Swan-Ganz-Catheter) showed an increase in both treatment groups during the first 24 hours (cf. post-text Table 14.2.7).

Table 9-8: Cardiac index at baseline and 4/24 hours post placement of Swan-Ganz-Catheter (full analysis set)

			Custodiol-N	Custodiol®
Cardiac index [L/min/m²]	Baseline	N	46	50
		Mean ± SD	2.1 ± 0.4	2.2 ± 0.5
		Range	1.3 – 3.3	1.4 – 3.6
	at 4 hrs	N	40	41
		Mean ± SD	2.4 ± 0.7	2.9 ± 0.7
		Range	1.2 – 4.3	1.4 – 5.3
	at 24 hrs	N	39	33
		Mean ± SD	2.8 ± 0.7	3.0 ± 0.6
		Range	1.6 – 5.2	2.0 – 4.5

Baseline = Placement of Swan-Ganz-Catheter
Abbreviations: hrs = Hours
Data source: Post-text Table 14.2.7

Figure 9-3: Course of cardiac index



Baseline = Placement of Swan-Ganz-Catheter
Abbreviations: hrs = hours
Source: Post-text Table 14.2.7, full analysis set

9.4.1.2.7 Requirement of defibrillation (postoperative period)

Only 1 patient (pat. no. 58, Custodiol® group) required 1 postoperative defibrillation (cf. post-text Table 14.2.9, FAS).

9.4.1.2.8 Requirement for intraaortic balloon pump (IABP)

No patient required the use of an intraaortic balloon pump within 5 days post opening of aortic cross clamp.

9.4.1.2.9 Blood pressure and heart rate

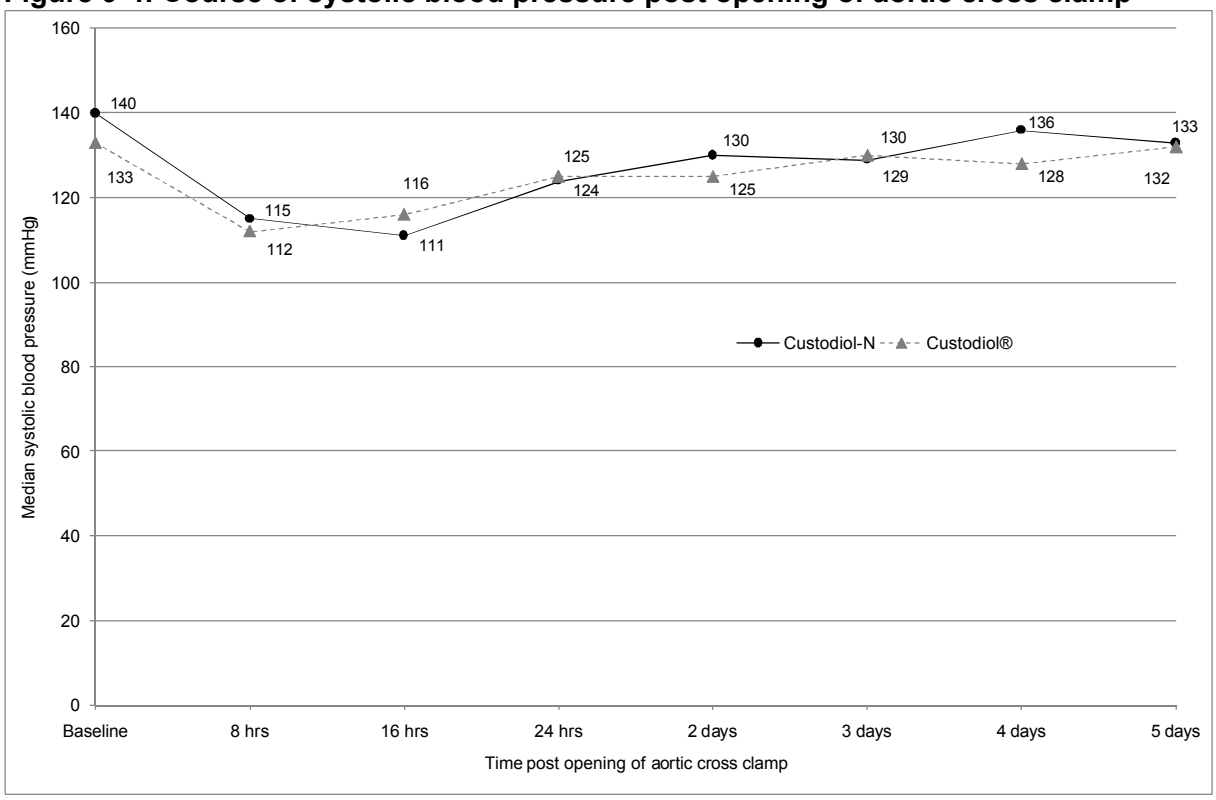
As displayed in Table 9-9, Figure 9-4 and Figure 9-5, the analysis of systolic and diastolic blood pressure revealed no relevant differences between the treatment groups. Median values showed a temporary decrease in the early postoperative phase (cf. post-text Table 14.2.6).

Table 9-9: Blood pressure at baseline and on Day 5 (full analysis set)

			Custodiol-N	Custodiol®
Systolic BP [mmHg]	Baseline	N	49	51
		Mean ± SD	139 ± 20	138 ± 23
		Range	100 - 190	83 - 200
	Day 5	N	49	51
		Mean ± SD	131 ± 17	132 ± 20
		Range	96 - 180	100 - 187
Diastolic BP [mmHg]	Baseline	N	49	51
		Mean ± SD	76 ± 13	77 ± 12
		Range	40 - 110	51 - 110
	Day 5	N	49	49
		Mean ± SD	72 ± 12	76 ± 15
		Range	44 - 110	49 - 129

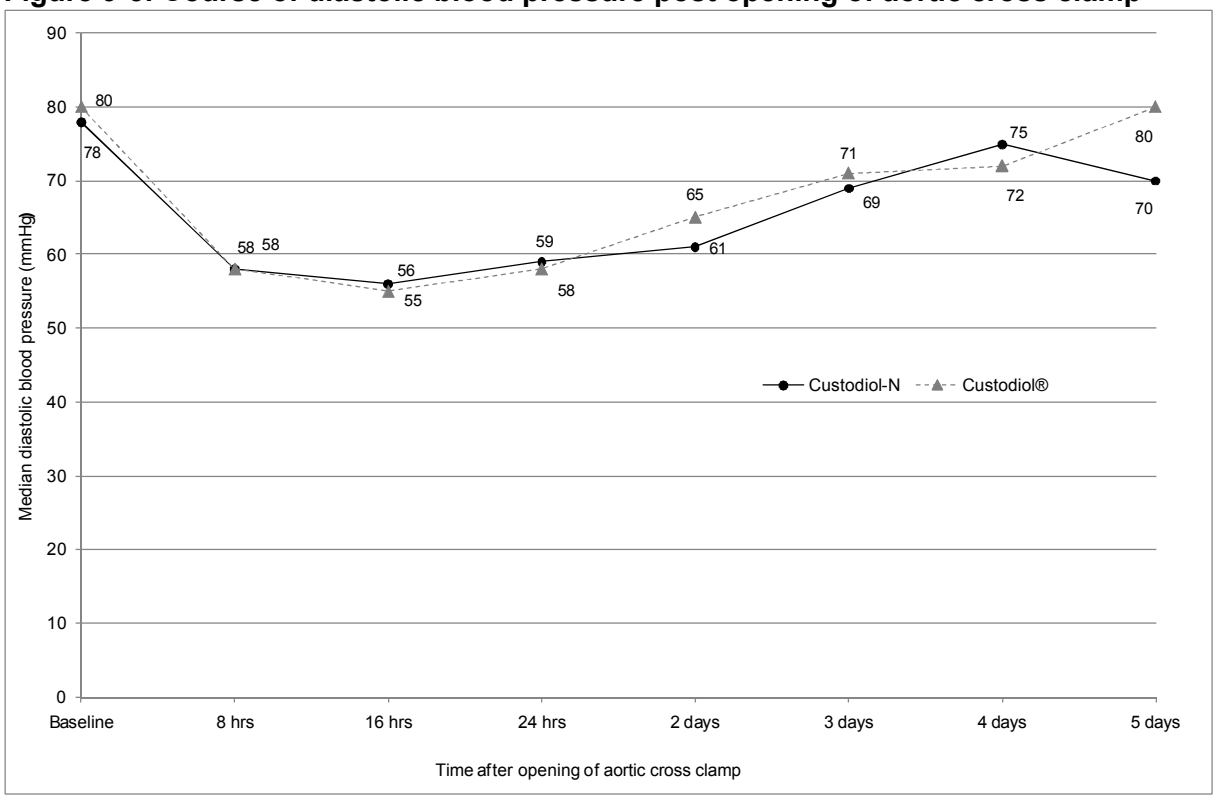
Abbreviations: BP = Blood pressure
Data source: Post-text Table 14.2.6

Figure 9-4: Course of systolic blood pressure post opening of aortic cross clamp



Please note non-linear scaling on x-axis. Abbreviations: hrs = hours
Source: Post-text Table 14.2.6, full analysis set

Figure 9-5: Course of diastolic blood pressure post opening of aortic cross clamp



Please note non-linear scaling on x-axis. Abbreviations: hrs = hours
Source: Post-text Table 14.2.6, full analysis set

The evaluation of heart rate revealed no meaningful changes during the study or relevant differences between the treatment groups. The mean heart rate at baseline was 74 ± 12 bpm (median: 72 bpm) in the Custodiol-N group and 73 ± 13 bpm (median: 70 bpm) in the Custodiol® group, the mean heart rate 5 days after the operation was 84 ± 11 bpm (median: 85 bpm) in the Custodiol-N group and 86 ± 13 bpm (median: 85 bpm) in the Custodiol® group (cf. post-text Table 14.2.6).

9.4.1.2.10 Length of surgical intensive care unit (SICU) stay

As shown in Table 9-10, the majority of patients in both treatment groups (79.6% in the Custodiol-N group and 82.0% in the Custodiol® group) were discharged from the SICU between 1 and 4 days after the operation. The duration of the SICU stay did not differ significantly between the treatment groups (cf. post-text Table 14.2.11, FAS). In the mean, patients in the Custodiol-N group were discharged from the SICU on Day 3 ± 2 (median: Day 3; range: Day 1 – Day 8) and in the Custodiol® group on Day 3 ± 5 (median: Day 2; range: Day 1 – Day 33; cf. post-text Table 14.2.10, FAS).

Table 9-10: Discharge from surgical intensive care unit (full analysis set)

Day of discharge	Custodiol-N N=49 n (%)	Custodiol® N=50 n (%)
Day1	14 (28.6)	19 (38.0)
Day 2	8 (16.3)	13 (26.0)
Day 3	7 (14.3)	6 (12.0)
Day 4	10 (20.4)	3 (6.0)
Day 5 or later[a]	10 (20.4)	9 (18.0)

[a] = Percentages calculated by the author.
Data source: Post-text Table 14.2.11

9.4.1.2.11 Duration of mechanical ventilation (intubation to extubation)

The mean duration of mechanical ventilation was overall comparable in the Custodiol-N group (25 ± 17 hours; median: 23 hours) and in the Custodiol® group (21 ± 17 hours; median: 19 hours) (p=0.070; Wilcoxon rank sum test; cf. Section 16.1.9) (cf. post-text Table 14.2.13, FAS).

9.4.1.2.12 Duration of surgical procedures

As summarized in Table 9-11, the median duration of the surgical procedures evaluated was similar in both treatment groups. Median cross clamp time was 41 minutes in the Custodiol-N group and 47 minutes in the Custodiol® group (cf. post-text Table 14.2.8).

Table 9-11: Duration of surgical procedures (full analysis set)

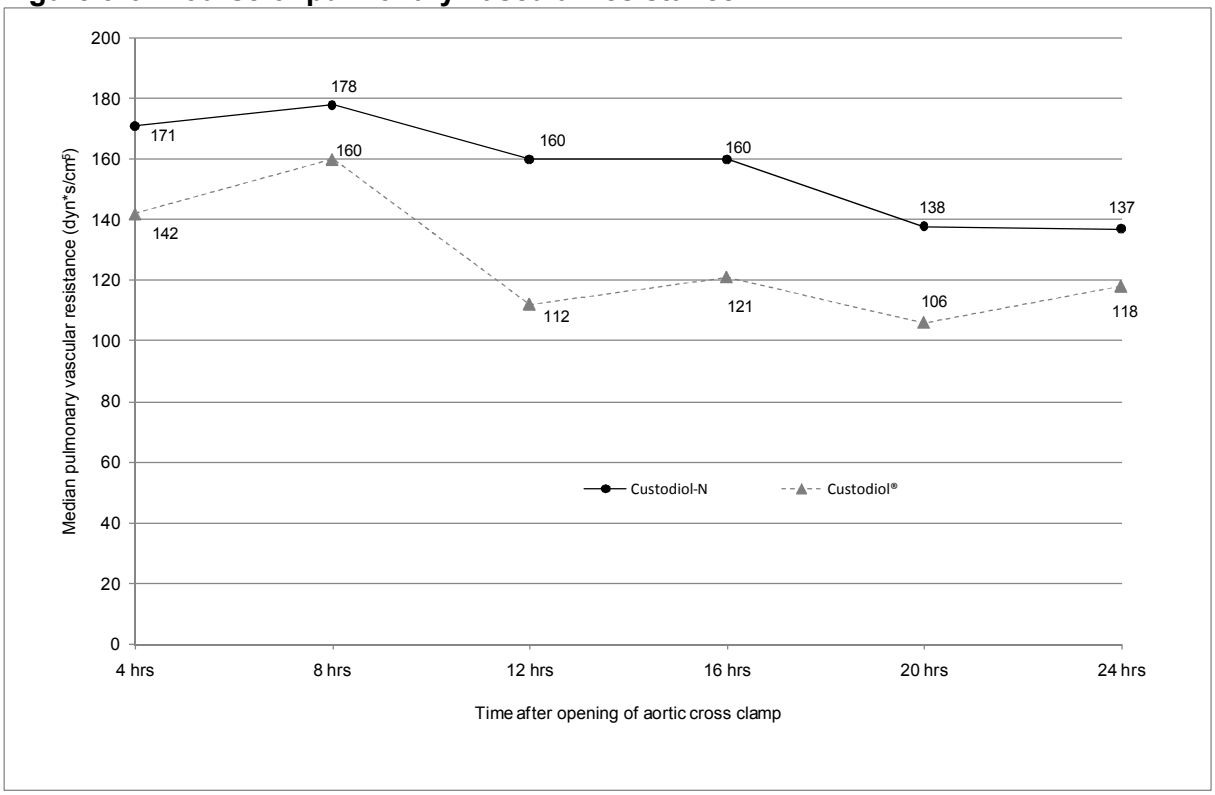
		Custodiol-N Time in h:min	Custodiol® Time in h:min
Start of anesthesia to cut	Median	1:23	1:19
	Range	0:36 – 2:15	0:57 – 2:36
Start of anesthesia to SGC	Median	0:40	0:40
	Range	0:0 – 3:05	0:15 – 2:20
Bypass on - off	Median	1:08	1:06
	Range	0:25 – 3:49	0:24 – 3:07
Cross clamp on - off	Median	0:41	0:47
	Range	0:23 – 1:52	0:17 – 1:33
Cut to skin closure	Median	3:07	3:09
	Range	1:50 – 5:40	1:40 – 5:30
Perfusion with cardioplegia solution	Median	0:07	0:07
	Range	0:04 – 1:18	0:03 – 1:44
Initiation of cardioplegic solution to cardiac arrest	Median	0:02	0:02
	Range	0:01 – 0:06	0:00 – 0:06
Cross clamp off to spontaneous cardiac activity	Median	0:01	0:01
	Range	0:00 – 0:06	0:00 – 0:09

Abbreviations: SGC = Swan-Ganz-Catheter
Data source: Post-text Table 14.2.8

9.4.1.2.13 Pulmonary and systemic vascular resistance

As shown in Figure 9-6, pulmonary vascular resistance showed slightly elevated median values at 4 hours post opening of the aortic cross clamp followed by a further slight increase at 8 hours and a subsequent decrease. Mean values decreased from 185 ± 147 dyn*s/cm^5 in the Custodiol-N group and 157 ± 118 dyn*s/cm^5 in the Custodiol® group at 4 hours (p=0.32) to 145 ± 87 dyn*s/cm^5 in the Custodiol-N group and 134 ± 77 dyn*s/cm^5 in the Custodiol® group at 24 hours (p=0.50; Wilcoxon rank sum test; cf. Section 16.1.9) (cf. post-text Table 14.2.6).

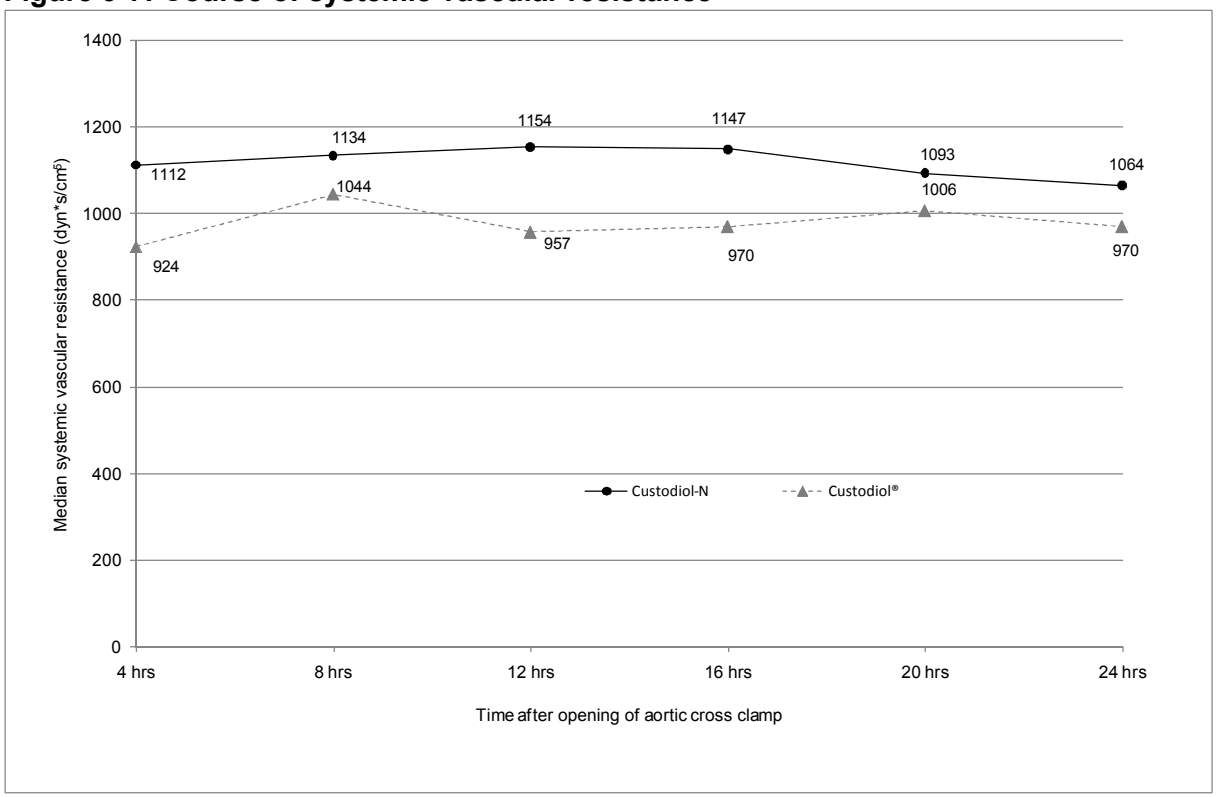
Figure 9-6: Course of pulmonary vascular resistance



p=0.32 at 4 hrs; p=0.59 at 8 hrs; p=0.32 at 12 hrs; p=0.19 at 16 hrs; p=0.11 at 20 hrs; p=0.50 at 24 hrs; Wilcoxon rank sum test (cf. Section 16.1.9).
Abbreviations: hrs = hours
Source: Post-text Table 14.2.6, full analysis set

As shown in Figure 9-7, systemic vascular resistance was within the physiological range and did not differ significantly between the treatment groups. No noteworthy changes were observed during the first 24 hours post opening of the aortic cross clamp in either treatment group. Mean values were 1316 ± 811 dyn*s/cm^5 in the Custodiol-N group and 1044 ± 459 dyn*s/cm^5 in the Custodiol® group at 4 hours and 1210 ± 399 dyn*s/cm^5 in the Custodiol-N group and 1025 ± 358 dyn*s/cm^5 in the Custodiol® group at 24 hours (cf. post-text Table 14.2.6).

Figure 9-7: Course of systemic vascular resistance



Abbreviations: hrs = hours
Source: Post-text Table 14.2.6, full analysis set

9.4.1.2.14 Cardiac arrhythmias (postoperative period)

The incidences of different forms of cardiac arrhythmia in the postoperative period are summarized in Table 9-12. Most common was atrial fibrillation occurring in 30% of all patients. (cf. post-text Table 14.2.14; FAS).

Table 9-12: Incidence of postoperative cardiac arrhythmias (full analysis set)

		Custodiol-N	Custodiol®	Total
	Number of episodes	N=49 n (%)	N=51 n (%)	N=100 n (%)
Atrial fibrillation	0	35 (71.4)	35 (68.6)	70 (70.0)
	1	12 (24.5)	7 (13.7)	19 (19.0)
	2	1 (2.0)	6 (11.8)	7 (7.0)
	3	1 (2.0)	3 (5.9)	4 (4.0)
Ventricular tachycardia	0	45 (91.8)	50 (98.0)	95 (95.0)
	1	3 (6.1)	1 (2.0)	4 (4.0)
	2	1 (2.0)	0 (0.0)	1 (1.0)
	3	0 (0.0)	0 (0.0)	0 (0.0)
Ventricular fibrillation	0	49 (100.0)	50 (98.0)	99 (99.0)
	1	0 (0.0)	1 (2.0)	1 (1.0)
	2	0 (0.0)	0 (0.0)	0 (0.0)
	3	0 (0.0)	0 (0.0)	0 (0.0)

Data source: Post-text Table 14.2.14

9.4.1.2.15 Laboratory parameters

Laboratory parameters are described in Section 10.4.

9.4.1.2.16 Repeated patient transfer to surgical intensive care unit (SICU)

Only 1 patient (pat. no. 32, Custodiol® group) was re-admitted to the SICU within 5 days post opening of the aortic cross clamp (cf. post-text Table 14.2.9).

9.4.1.2.17 Mortality any time during post-op through Day 30

One patient in each treatment group died during the study up to day 30 (cf. post-text Table 14.2.12). One further patient in the Custodiol® group died on day 43 (cf. post-text Table 14.3.2.1).

9.4.2 Statistical/analytical issues

9.4.2.1 Adjustments for covariates

Refer to Section 9.4.2.4 since study center was the only variable adjusted for.

9.4.2.2 Handling of dropouts or missing data

As all patients receiving cardioplegic solution had their blood sampled up to 24 hours post opening of the aortic cross-clamp, drop-outs (other than for patients' deaths) have not been an issue.

Missing values were only imputed for calculation of CK-MB and TroponinT after re-opening of the aortic cross-clamp.

Missing values at measurement points 8, 12, 16, and 20 hours post opening of the aortic cross-clamp were ignored for calculating the area under the curve, leading to linear interpolation between neighboring time points.

For missing data at 4 h and 24 h time points, the following method was applied. For each time point, the empirical distribution of the laboratory parameter was obtained. For the non-missing value time points, the percentile of the current point within the time-point specific empirical distribution was calculated. Out of all percentile values for "non-missing time points" the median percentile was obtained. The percentile was obtained from all empirical distributions for the "missing time points" and those values were imputed for the missing time points.

For the handling of CK-MB values measured by concentration rather than by enzymatic activity, see Section 7.8.3.

9.4.2.3 Interim analyses and data monitoring

After 10 patients were enrolled into a Custodiol-N only treated cohort, safety analyses were performed. The results of these analyses were compiled and commented to a report, which was sent to the Ethics Committee in Heidelberg.

The interim analysis after enrollment of 50 patients, carried out according to plan as described in 7.7.7, yielded test statistics of 3.64 for the FAS and 3.60 for the PP set. These values were higher than the critical values of the tests for non-inferiority at the 30% margin, and therefore would have been sufficient to reject the null hypothesis of inferiority of Custodiol-N by 30% or more. Therefore, it was decided that no additional patients were required to answer the efficacy part of the study. In order to enroll a sufficient number of patients to answer the safety question, it had been planned that at least 50 more patients entered the study. Thus, the study continued until 100 patients were enrolled.

9.4.2.4 Multicenter Studies

The study was carried out in 4 study centers with a clear preponderance of patients included in the study center in Heidelberg (FAS: 82 patients in Heidelberg and 19 patients in all other sites). In order to assess a possible center effect, the study center was included into the model in a secondary analysis (see also Section 7.7.5). When evaluating the CK-MB and troponin T $AUC_{4\text{-}24h}$, the factor study center seemed to have an effect on troponin T $AUC_{4\text{-}24h}$ with markedly higher median values in Heidelberg in both treatment groups. The largest difference was observed between the study centers Jena and Heidelberg (median troponin T $AUC_{4\text{-}24h}$: 6280 h*pg/mL in Jena compared with 13654 h*pg/mL in Heidelberg for the Custodiol-N group and 5590 h*pg/mL in Jena compared with 13318 h*pg/mL in Heidelberg for the Custodiol® group). However, taking into consideration the different sample sizes (3 patients per treatment group in Jena vs. 41 patients in Heidelberg), this finding needs to be interpreted with care; cf. post-text Table 14.2.3).

Results of the subgroup analysis of the primary endpoint differentiating between the study center Heidelberg and all other study centers are summarized in Table 9-13. The low sample size in the "all other sites" subgroup resulted in a high variability of values as indicated by the large confidence intervals. For CK-MB $AUC_{4\text{-}24h}$, the distance to the non-inferiority margin was more pronounced in the Heidelberg subgroup (36.0%, $p<0.0001$) than in the "all other

sites" subgroup (28.0%, p=0.1705) (FAS). The same was observed for the secondary endpoint troponin T, with a distance to the 30% non-inferiority margin of 24.6% (p=0.0009) in the Heidelberg subgroup and 17.3% (p=0.2937) in the "all other sites" subgroup (post-text Table 14.2.2).

Table 9-13: Sensitivity analysis: Treatment effect on CK-MB AUC_{4-24h} and troponin T AUC_{4-24h} differentiated by study center (full analysis set)

Parameter	**% Difference** (Custodiol® minus Custodiol-N)[a]	**[95% CI]**	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 30%)	**P-value** (one-sided) (H_0 of inferiority of Custodiol-N by 0%)	**Study site**
CK-MB	6.0	[-6.3; 19.9]	<0.0001	0.1759	Heidelberg
	-2.0	[-42.0; 65.7]	0.1705	0.5320	Other sites
Troponin T	-5.4	[-16.8; 7.5]	0.0009	0.8039	Heidelberg
	-12.7	[-46.3; 41.8]	0.2937	0.7205	Other sites

Note: Sensitivity analysis → P-values have to be interpreted descriptively.
[a] = Antilog of difference of mean log(AUC) in Custodiol® patients minus mean log(AUC) in Custodiol-N patients.
Abbreviations: CI = Confidence interval; H_0 = Null hypothesis
Data source: Post-text Table 14.2.2

9.4.2.5 Multiple Comparison/Multiplicity

Only the 4-24 h AUC for CK-MB was used to address the primary hypothesis. Both FAS and PP sets were used for the test, but as both tests had to reject the null hypothesis in order to answer the efficacy question, no augmentation of type I error was brought about.

9.4.2.6 Examination of Subgroups

Refer to Section 9.4.2.4. No further subgroup analyses were performed.

9.4.3 Tabulation of individual response data

Individual CK-MB measurements are provided in post-text Listing 16.2.6.

9.4.4 Drug dose, drug concentration, and relationships to response

Drug dose or concentration relationship to response was not analyzed.

9.4.5 Drug-drug and drug-disease interactions

Drug-drug and drug-disease interactions were not analyzed.

9.4.6 Efficacy conclusions

The primary analysis statistically confirmed non-inferiority of Custodiol-N compared with Custodiol® as determined by the CK-MB AUC_{4-24h} (FAS: Percentage difference [Custodiol® minus Custodiol-N] of 2.7; 95% CI: [-8.3; 15.0]; p< 0.0001 [30% margin]; PP set: Percentage difference of 2.5; 95% CI: [-9.2; 15.8]; p< 0.0001 [30% margin]).

The result of the primary analysis was supported by the results of the following secondary analyses:

- Treatment effect on troponin T AUC_{4-24h}: Percentage difference [Custodiol® minus Custodiol-N] of -9.2; 95% CI: [-19.1; 1.9]; p=0.0027 (30% margin) (FAS).
- Treatment effect on CK-MB peak values: Percentage difference of 10.9; 95% CI: [-0.6; 23.9]; p< 0.0001 (30% margin); evaluation at the 0% margin (p=0.0322; FAS) showed more favorable results under Custodiol-N. This was even more pronounced in the complete case analysis where the extrapolated data from the study center in Jena were omitted (p=0.0239).
- Treatment effect on troponin T peak values: Percentage difference of -3.0; 95% CI: [-13.0; 8.2]; p< 0.0001 (FAS).

In a subgroup analysis differentiated by study center, the distance to the non-inferiority margin for CK-MB AUC_{4-24h} was more pronounced in the Heidelberg subgroup (36.0%, p<0.0001) than in the "all other sites" subgroup (28.0%, p=0.1705) (FAS). The same was observed for the secondary endpoint troponin T, with a distance to the 30% non-inferiority margin of 24.6% (p=0.0009) in the Heidelberg subgroup and 17.3% (p=0.2937) in the "all other sites" subgroup.

Further secondary analyses showed (FAS):

- a similar course of CK-MB values post opening of the aortic cross clamp in both treatment groups with increased median values at 4 hours (29 U/L in the Custodiol-N group and 31 U/L in the Custodiol® group) and a further slight increase at 8 hours (32 U/L in the Custodiol-N group and 36 U/L in the Custodiol® group) followed by a clear decrease at Day 2 (19 U/L in both treatment groups). During the first 24 hours, median values in the Custodiol-N group were slightly lower than in the Custodiol® group.
- a similar course of troponin T values post opening of the aortic cross clamp in both treatment groups with increased median values at 4 hours (638 pg/mL in the

Custodiol-N group and 537 pg/mL in the Custodiol® group) and a further increase at 8 hours (810 pg/mL in the Custodiol-N group and 828 pg/mL in the Custodiol® group) followed by a gradual and constant decrease (200 pg/mL in the Custodiol-N group and 217 pg/mL in the Custodiol® group at Day 5). At 4 hours, median values in the Custodiol-N group were higher than in the Custodiol® group.

- a similar mean/median cardiac index in both treatment groups at baseline (placement of Swan-Ganz-Catheter) and an increase in both treatment groups during the first 24 hours.
- no relevant differences between the treatment groups with respect to catecholamine demand.
- no requirement of intraaortic balloon pump within 5 days post opening of aortic cross clamp in either treatment group.
- overall comparable values for pulmonary vascular resistance within the first 24 hours post opening of the aortic cross clamp. Mean values decreased from 185 ± 147 dyn*s/cm^5 in the Custodiol-N group and 157 ± 118 dyn*s/cm^5 in the Custodiol® group at 4 hours (p=0.32) to 145 ± 87 dyn*s/cm^5 in the Custodiol-N group and 134 ± 77 dyn*s/cm^5 in the Custodiol® group at 24 hours (p=0.50; Wilcoxon rank sum test).
- no noteworthy changes in systemic vascular resistance within the first 24 hours post opening of the aortic cross clamp in either treatment group.
- no unexpected changes during the study or relevant differences between the treatment groups with respect to blood pressure or heart rate.
- an overall comparable mean duration of mechanical ventilation in the Custodiol-N group (25 ± 17 hours; median: 23 hours) and in the Custodiol® group (21 ± 17 hours; median: 19 hours) (p=0.070; Wilcoxon rank sum test.
- no relevant difference between the treatment groups with respect to the duration of the SICU stay.
- atrial fibrillation to be the most common form of postoperative cardiac arrhythmia (reported in 30% of all patients).
- requirement of postoperative defibrillation in only 1 patient in the Custodiol® group.

- re-admission to the SICU within 5 days post opening of the aortic cross clamp in only 1 patient in the Custodiol® group.
- no difference in mortality between the treatment groups. One patient in each treatment group died during the study up to day 30. One further patient in the Custodiol® group died on day 43.

10 SAFETY EVALUATION

10.1 Extent of Exposure

All of the patients who received study treatment were included into the safety population. These were 59 patients in the Custodiol-N group (including the 10 patients of the "Custodiol-N only treated cohort" in the first stage of the study) and 52 patients in the Custodiol® group (cf. post-text Table 14.1.1).

The median perfusion time with cardioplegia solution was 7 minutes in both treatment groups (cf. post-text Table 14.2.8).

10.2 Adverse Events (AEs)

10.2.1 Brief summary of adverse events

An overview of AEs is given in Table 10-1. As could be expected in this patient population under the given circumstances, a high proportion of patients experienced AEs. The incidence of AEs was slightly higher in the Custodiol® group than in the Custodiol-N group. Drug-related or serious AEs were infrequently reported.

Table 10-1: Overview of adverse events (AEs) (safety set)

	Custodiol-N (N=59) n (%)	Custodiol® (N=52) n (%)	Total (N=111) n (%)
Patients with			
any AE	47 (79.7)	45 (86.5)	92 (82.9)
drug-related AE (probable relationship to SM)	1 (1.7%)	2 (3.8)	3 (2.7)
any serious AE (SAE)[a]	1 (1.7%)	3 (5.8)	4 (3.6)
Deaths[b]	1 (1.7%)	2 (3.8)	3 (2.7)

[a] = Percentages calculated by the author.
[b] = Including patient no. 1082 who died of ventricular fibrillation occurring outside of the reporting period for AEs/SAEs.
Abbreviations: SM=study medication
Data source: Post-text Tables 14.3.1.3 and 14.3.2.1; post-text Listing 16.2.1.1

10.2.2 Display of adverse events

As shown in Table 10-2, most frequently reported were "cardiac disorders" and "injury, poisoning & procedural complications". Overall, the incidences of AEs in the different MedDRA System Organ Classes were comparable in the treatment groups. A difference > 10% was observed for psychiatric disorders (5.1% in the Custodiol-N group versus 15.4%

in the Custodiol® group). Psychiatric disorders were mostly cases of insomnia/sleep disorder (cf. post-text Tables 14.3.1.3 and 14.3.1.1).

Table 10-2: Adverse events by MedDRA PSOC (safety set)

	Custodiol-N (N=59) n (%)	Custodiol® (N=52) n (%)	Total (N=111) n (%)
Total	47 (79.7)	45 (86.5)	92 (82.9)
MedDRA PSOC			
Cardiac disorders	28 (47.5)	23 (44.2)	51 (45.9)
Injury, poisoning & procedural complications	12 (20.3)	15 (28.8)	27 (24.3)
Blood and lymphatic system disorders	8 (13.6)	6 (11.5)	14 (12.6)
Gastrointestinal disorders	8 (13.6)	10 (19.2)	18 (16.2)
Respiratory, thoracic and mediastinal disorders	7 (11.9)	5 (9.6)	12 (10.8)
Infections and infestations	6 (10.2)	8 (15.4)	14 (12.6)
Investigations	5 (8.5)	6 (11.5)	11 (9.9)
General disorders and administration site conditions	4 (6.8)	2 (3.8)	6 (5.4)
Psychiatric disorders	3 (5.1)	8 (15.4)	11 (9.9)
Nervous system disorders	2 (3.4)	0	2 (1.8)
Renal and urinary disorders	2 (3.4)	0	2 (1.8)
Metabolism and nutrition disorders	1 (1.7)	0	1 (0.9)
Musculoskeletal and connective tissue disorders	1 (1.7)	1 (1.9)	2 (1.8)
Skin and subcutaneous tissue disorders	1 (1.7)	1 (1.9)	2 (1.8)
Vascular disorders	1 (1.7)	0	1 (0.9)
Immune system disorders	0	1 (1.9)	1 (0.9)

Presented in descending order of frequency in Custodiol-N group and then alphabetically.
Abbreviations: MedDRA PSOC=Medical Dictionary for Regulatory Activities, Primary System Organ Class
Data source: Post-text Table 14.3.1.3

Most common AEs (reported in > 1 patient in either treatment group) by MedDRA Preferred Term are shown in Table 10-3 (cf. post-text Tables 14.3.1.1 and 14.3.1.2). Most frequently reported (20 events in the Custodiol-N group and 26 events in the Custodiol® group) and with similar incidence in both treatment groups was atrial fibrillation. Overall, there were no relevant differences between the treatment groups with respect to the incidence of individual AEs. The cases of "anesthetic complication neurological" referred to postoperative delirium/disorientation (cf. post-text Listing 16.2.7.1). One case of acute myocardial infarction (original term: N-STEMI [non-ST elevation myocardial infarction]; pat. no. 58) was reported in the Custodiol® group. The event was considered not to be related to the study medication and the outcome was "recovered". One case of cardiac arrest was reported in each treatment group. One of the cases was fatal (pat. no 1035; Custodiol® group); the other patient (no. 1082; Custodiol-N group) recovered. Neither case was considered to be related to the study medication (cf. post-text Listing 16.2.7.1).

Table 10-3: Most frequent (> 1 pat. affected in either treatment group) adverse events by MedDRA Preferred Term (safety set)

	Custodiol-N (N=59) n (%)	Custodiol® (N=52) n (%)	Total (N=111) n (%)
Total	47 (79.7)	45 (86.5)	92 (82.9)
MedDRA Preferred Term			
Atrial fibrillation	17 (28.8)	16 (30.8)	33 (29.7)
Anemia	8 (13.6)	6 (11.5)	14 (12.6)
Anesthetic complication neurological	5 (8.5)	6 (11.5)	11 (9.9)
Pleural effusion	5 (8.5)	2 (3.8)	7 (6.3)
Nausea	4 (6.8)	3 (5.8)	7 (6.3)
Ventricular tachycardia	4 (6.8)	1 (1.9)	5 (4.5)
Vomiting	4 (6.8)	5 (9.6)	9 (8.1)
Wound complication	4 (6.8)	4 (7.7)	8 (7.2)
Ventricular extrasystoles	3 (5.1)	1 (1.9)	4 (3.6)
Cystitis	2 (3.4)	5 (9.6)	7 (6.3)
Pain	2 (3.4)	0	2 (1.8)
Pericardial effusion	2 (3.4)	5 (9.6)	7 (6.3)
Sleep disorder	2 (3.4)	4 (7.7)	6 (5.4)
Transaminases increased	2 (3.4)	1 (1.9)	3 (2.7)
Urinary tract infection	2 (3.4)	2 (3.8)	4 (3.6)
Ventricular fibrillation	2 (3.4)	1 (1.9)	3 (2.7)
Wound dehiscence	2 (3.4)	0	2 (1.8)
Blood creatine phosphokinase MB increased	1 (1.7)	2 (3.8)	3 (2.7)
Insomnia	1 (1.7)	2 (3.8)	3 (2.7)
Post procedural hemorrhage	0	2 (3.8)	2 (1.8)
Psychotic disorder	0	2 (3.8)	2 (1.8)

Presented in descending order of frequency in Custodiol-N group and then alphabetically.
Abbreviations: MedDRA=Medical Dictionary for Regulatory Activities
Data source: Post-text Table 14.3.1.1

10.2.3 Analysis of adverse events

10.2.3.1 Drug-related adverse events

The vast majority of patients (74.6% in the Custodiol-N group and 76.9% in the Custodiol® group) experienced AEs which were considered not to be related to the study medication. AEs with probable relationship to the study medication were reported in 1 patient (1.7%) in the Custodiol-N group and in 2 patients (3.8%) in the Custodiol® group.

AEs with probable relationship to the study medication in the Custodiol-N group were:

- atrial fibrillation and pleural effusion in patient no. 35.

AEs with probable relationship to the study medication in the Custodiol® group were.

- psychotic disorder in patient no. 32 and
- 2 events of atrial fibrillation and 1 event of respiratory failure in patient no. 33.

All of these events were of moderate severity and all except pleural effusion, where the patient was recovering, had resolved by the end of the reporting period.

Events that were considered to be unlikely related to the study medication were reported in 2 patients (3.4%) in the Custodiol-N group and in 3 patients (5.8%) in the Custodiol® group. These were, in the Custodiol-N group, urinary tract infection (2 patients) and pleural effusion, and in the Custodiol® group ventricular tachycardia, urinary tract infection, postoperative anemia, pneumothorax, increased white blood cell count and subcutaneous emphysema (cf. post-text Table 14.3.1.3 and post-text Listing 16.2.7.1).

10.2.3.2 Severity of adverse events

The majority of patients experienced AEs of maximally mild (54.2% in the Custodiol-N group and 51.9% in the Custodiol® group) or moderate (18.6% in the Custodiol-N group and 30.8% in the Custodiol® group) severity. Severe AEs were reported in 4 patients (6.8%) in the Custodiol-N group and in 2 patients (3.8%) in the Custodiol® group (cf. post-text Table 14.3.1.4 and post-text Listing 16.2.7.1).

Severe AEs in the Custodiol-N group were:

- "gamma-glutamyltransferase increased" in patient no. 1 (not related to the study medication; outcome: recovering)
- "anesthetic complication neurological" (original term: postop delirium; not related to the study medication; outcome: recovered) in patient no. 79.
- ventricular fibrillation (not related to the study medication; outcome: recovered) in patient no. 1022
- cardiac arrest (serious; not related to the study medication; outcome: recovered) in patient no. 1082.

Severe AEs in the Custodiol® group were:

- pneumothorax (serious) and subcutaneous emphysema (both assessed as unlikely related to the study medication; outcome: fatal) in patient no. 1011.
- cardiac arrest (serious; not related to the study medication; outcome: fatal) in patient no. 1035.

10.2.4 Listing of adverse events by patient

A by-patient listing of all adverse events is provided in Section 16.2.7.

10.3 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

10.3.1 Listing of deaths, other serious adverse events, and other significant adverse events

10.3.1.1 Deaths

Three patients (1 in the Custodiol-N group and 2 in the Custodiol® group) died during the study:

Custodiol-N group:

- Patient no. 1082 died on Day 7 (cf. post-text Listing 16.2.1.1). The course of death was ventricular fibrillation (data on file). Please note that this event occurred outside of the reporting period for AEs/SAEs (up to Day 5 ± 1).

Custodiol® group:

- Patient no. 1011 died on Day 43 (cf. post-text Table 14.3.2.1) of severe respiratory insufficiency due to progressive pneumothorax with skin emphysema (cf. SAE narrative in Section 15). The pneumothorax had occurred on Day 3. The patient was thoracotomized. The event was considered as being unlikely related to the study medication.
- Patient no. 1035 died of cardiac arrest on Day 0. The event was considered not to be related to the study medication (cf. post-text Table 14.3.2.1 and post-text Listing 16.2.7.1).

10.3.1.2 All Serious Adverse Events

The listing of SAEs is shown in post-text Table 14.3.2.1. See also post-text Listing 16.2.7.2 and SAE narratives in Section 15.

SAEs which were reported for a total of 4 patients (1 in the Custodiol-N group and 3 in the Custodiol® group) and none of which were considered as being related to the study

medication, are summarized in Table 10-4. Two SAEs (pneumothorax and cardiac arrest) both occurring in the Custodiol® group had a fatal outcome.

Table 10-4: Serious adverse events (safety set)

Treatment group	Pat. no.	Start day	End day	SAE (preferred term)	Severity	Relationship to SM	Outcome
Custodiol®	32	6	27	pleural effusion	moderate	not related	recovered
	1011	3	43	pneumothorax	severe	unlikely related	fatal
	1035	0	-	cardiac arrest	severe	not related	fatal
Custodiol-N	1082	2	3	cardiac arrest	severe	not related	recovered

Abbreviations: SM=Study medication
Data source: Post-text Listings 16.2.7.1 and 16.2.7.2

10.3.1.3 Other Significant Adverse Events

Not applicable.

10.3.2 Narratives of deaths, other serious adverse events, and certain other significant adverse events

Detailed subject narratives are provided in Section 15.

10.3.3 Analysis and discussion of deaths, other serious adverse events, and other significant adverse events

None of the SAEs or deaths reported in this study was considered as being related to the study medication.

10.4 Clinical Laboratory Evaluation

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

The tabular listings by patient of all safety-related laboratory values are presented in post-text Listing 16.2.8. In addition, a listing by-patient of all abnormal values is presented in post-text Table 14.3.3.5 and 14.3.3.6.

10.4.2 Evaluation of each laboratory parameter

10.4.2.1 Laboratory Values over Time

Laboratory parameters over time are shown in post-text Tables 14.3.3.1 (blood samples) and 14.3.3.2/3 (urinalysis). A summary of selected parameters is provided in Table 10-5. Mean post-baseline values showed a decrease in hemoglobin and related parameters, a temporary decrease in platelets, a temporary increase in white blood cells, a pronounced increase in C-reactive protein, a clear increase in GGT, and a slight increase in LDH, AST and ALT. No relevant differences were observed between the treatment groups.

The course of CK-MB and troponin T is presented in Section 9.4.1.2.3 and 9.4.1.2.4.

Table 10-5: Course of laboratory values over time (safety set)

		Custodiol-N		Custodiol®	
Parameter	**Time**	**n**	**Mean ± Std**	**n**	**Mean ± Std**
Hemoglobin [g/dL]	Baseline	59	13.6 ± 1.5	51	13.9 ± 1.4
	Surgery + ICU	59	11.2 ± 1.4	50	10.9 ± 1.3
	Follow-up (Day 5)	59	10.9 ± 1.3	51	10.7 ± 1.1
White blood cells [/nL]	Baseline	59	8.2 ± 2.2	51	8.1 ± 2.3
	Surgery + ICU	59	11.6 ± 3.5	48	11.6 ± 2.8
	Follow-up (Day 5)	59	8.9 ± 2.6	51	9.1 ± 3.4
Platelets [/nL]	Baseline	59	257.6 ± 72.2	51	262.0 ± 65.7
	Surgery + ICU	59	168.6 ± 58.1	49	166.9 ± 53.0
	Follow-up (Day 5)	59	246.3 ± 73.6	51	253.8 ± 75.4
Creatinine [mg/dL]	Baseline	59	0.9 ± 0.3	52	0.9 ± 0.3
	Surgery + ICU	58	1.0 ± 0.4	49	1.0 ± 0.5
	Follow-up (Day 5)	59	0.9 ± 0.3	51	1.0 ± 0.3
Potassium [mmol/L]	Baseline	59	4.1 ± 0.4	52	4.0 ± 0.3
	Surgery + ICU	57	4.7 ± 0.4	48	4.6 ± 0.6
	Follow-up (Day 5)	59	4.3 ± 0.4	51	4.3 ± 0.5
INR	Baseline	58	1.0 ± 0.1	52	1.0 ± 0.1
	Surgery + ICU	57	1.1 ± 0.1	48	1.1 ± 0.1
	Follow-up (Day 5)	56	1.0 ± 0.1	49	1.0 ± 0.1
aPTT [sec]	Baseline	59	25.5 ± 3.5	52	25.8 ± 4.5
	Surgery + ICU	57	31.7 ± 6.3	48	30.7 ± 7.8
	Follow-up (Day 5)	56	27.4 ± 5.5	48	29.5 ± 9.8
C-reactive protein [mg/L]	Baseline	59	6.6 ± 8.8	51	9.2 ± 12.8
	Surgery + ICU	57	254.1 ± 311.2	47	234.4 ± 286.7
	Follow-up (Day 5)	58	104.1 ± 96.4	51	113.6 ± 122.8
GGT [U/L]	Baseline	59	52.2 ± 52.1	52	66.5 ± 101.5
	Surgery + ICU	50	43.8 ± 43.2	40	44.5 ± 34.8
	Follow-up (Day 5)	55	147.3 ± 137.3	44	133.2 ± 123.8
Lactate dehydrogenase [U/L]	Baseline	52	238.8 ± 61.9	46	231.4 ± 68.8
	Surgery + ICU	51	283.1 ± 64.9	38	292.6 ± 79.6
	Follow-up (Day 5)	43	280.6 ± 62.5	45	291.9 ± 70.4
AST [U/L]	Baseline	55	31.0 ± 14.9	48	31.4 ± 17.2
	Surgery + ICU	53	52.9 ± 29.2	43	51.6 ± 28.3
	Follow-up (Day 5)	59	49.4 ± 28.5	49	43.8 ± 35.5
ALT [U/L]	Baseline	59	35.3 ± 19.8	52	38.3 ± 26.0
	Surgery + ICU	51	29.1 ± 15.9	40	32.2 ± 23.6
	Follow-up (Day 5)	55	54.3 ± 40.9	45	46.6 ± 37.9

Abbreviations: ALT=alanine aminotransferase; aPTT=activated partial thromboplastin time; AST=aspartate aminotransferase; GGT= Gamma-glutamyl transferase; ICU=Intensive care unit; INR=international normalised ratio; Std=standard deviation
Data source: Post-text Table 14.3.3.1

10.4.2.2 Individual Patient Changes

As could be expected under the circumstances of cardiac surgery, laboratory values outside of the normal range were common in this study. Shifts from normal baseline values to values outside of the normal range during the study were most frequently observed for (cf. post-text Table 14.3.3.4):

- Hematocrit (89.2% in the Custodiol-N group / 95.2% in the Custodiol® group)
- Hemoglobin (93.9% in the Custodiol-N group / 95.1% in the Custodiol® group)
- Red blood cells (94.1% in the Custodiol-N group / 97.6% in the Custodiol® group)
- Cardiac troponin T (100.0% in the Custodiol-N group / 92.5% in the Custodiol® group)
- Total creatine kinase (88.1% in the Custodiol-N group / 87.2% in the Custodiol® group)
- C-reactive protein (100.0% in the Custodiol-N group / 100.0% in the Custodiol® group)
- LDH (79.3% in the Custodiol-N group / 78.8% in the Custodiol® group)
- Total protein (78.3% in the Custodiol-N group and 81.6% in the Custodiol® group)

[Percentages based on number of patients with normal baseline values.]

Differences between the treatment groups with respect to shifts from normal to abnormal values were observed for the parameters:

- Chloride (20.0% in the Custodiol-N group / 9.5% in the Custodiol® group)
- Glucose (56.3% in the Custodiol-N group / 68.0% in the Custodiol® group)
- Aspartate aminotransferase (AST) (59.5% in the Custodiol-N group / 46.3% in the Custodiol® group)

10.4.2.3 Individual Clinically Significant Abnormalities

Laboratory abnormalities were not evaluated with regard to clinical significance.

10.5 Vital Signs, Physical Findings, and Other Observations Related to Safety

Please refer to Section 9.4.1.2.9.

10.6 Safety Conclusions

The safety evaluation did not reveal any relevant differences between the treatment groups. Drug-related or serious AEs were infrequently reported. None of the SAEs or deaths in this study was reported to be related to the study medication.

11 OTHER VARIABLES AND EVALUATIONS

Not applicable.

12 DISCUSSION AND OVERALL CONCLUSIONS

This prospective, randomized, double blind, multicenter Phase III study was conducted to compare the cardioprotective effects and safety of 2 cardioplegic solutions, Custodiol® and Custodiol-N in patients undergoing cardiopulmonary bypass for coronary artery bypass surgery. The study was carried out in 3 steps. After safety analyses were performed in 10 patients who were enrolled into a "Custodiol-N only treated cohort", the study was continued and a further 50 patients were enrolled and randomized to either Custodiol-N or Custodiol®. A planned interim analysis in these 50 patients showed results which would have been sufficient to show non-inferiority of Custodiol-N compared to Custodiol® at the planned 30% margin. Thus, it was decided only to continue recruitment until the sample size of 100 patients considered necessary for safety analyses had been reached. The FAS comprised 49 patients in the Custodiol-N group and 52 patients in the Custodiol® group, and the PP set 43 patients in the Custodiol-N group and 44 patients in the Custodiol® group.

Apart from higher rates of tobacco abuse, angina pectoris and hypercholesterolemia in the Custodiol-N group, demography and baseline characteristics were overall comparable between the treatment groups. Approximately 80% of patients were men. The mean age was 68.1 ± 8.0 years in the Custodiol-N group and 66.4 ± 8.5 years in the Custodiol® group. In both treatment groups approximately half of the patients presented with NYHA class II. The median cross clamp time was 41 minutes in the Custodiol-N group and 47 minutes in the Custodiol® group.

In the primary analysis, the cardioprotective effects of Custodiol-N and Custodiol® were compared using the CK-MB $AUC_{4\text{-}24h}$ as marker for myocardial damage. Based on the pre-defined 30% non-inferiority margin, non-inferiority of Custodiol-N compared with Custodiol® was statistically confirmed (FAS: Percentage difference [Custodiol® minus Custodiol-N] of 2.7; 95% CI: [-8.3; 15.0]; p< 0.0001; PP set: Percentage difference of 2.5; 95% CI: [-9.2; 15.8]; p< 0.0001).

The result of the primary analysis was supported by a secondary analysis which evaluated the treatment effect on the basis of CK-MB peak values. With a percentage difference of 10.9 (95% CI: [-0.6; 23.9]; p< 0.0001 [30% margin]) evaluation at the 0% margin (p=0.0322; FAS) showed more favorable results under Custodiol-N. This was even more pronounced in the complete case analysis where the extrapolated data from the study center in Jena were omitted (p=0.0239). Correspondingly, the course of CK-MB which showed the highest median values between 8 and 24 hours post opening of the aortic cross clamp, revealed a tendency towards slightly lower values in the Custodiol-N group during the first 24 hours.

Evaluation of the troponin T $AUC_{4\text{-}24h}$ (percentage difference of -9.2; 95% CI: [-19.1; 1.9]; p=0.0027 [FAS]) and troponin T peak values (percentage difference of -3.0; 95% CI: [-13.0; 8.2]; p<0.0001 [FAS]) supported non-inferiority of Custodiol-N compared to Custodiol® at the 30% non-inferiority margin.

It should be noted that there was an imbalance in the number of patients enrolled in the 4 study centers with a preponderance of patients included in Heidelberg (FAS: 82 patients in Heidelberg and 19 patients in all other sites). A subgroup analysis differentiated by study center showed a high variability of values in the "all other sites" subgroup. The distance to the non-inferiority margin for CK-MB $AUC_{4\text{-}24h}$ was more pronounced in the Heidelberg subgroup (36.0%, p<0.0001) than in the "all other sites" subgroup (28.0%, p=0.1705) (FAS). The same was observed for the secondary endpoint troponin T, with a distance to the 30% non-inferiority margin of 24.6% (p=0.0009) in the Heidelberg subgroup and 17.3% (p=0.2937) in the "all other sites" subgroup.

Secondary analyses showed overall comparable results in both treatment groups for pulmonary and systemic vascular resistance, catecholamine demand, blood pressure, heart rate, requirement of postoperative defibrillation, duration of mechanical ventilation, re-admission to the SICU, duration of SICU stay and mortality. The incidence of cardiac arrhythmias did not differ between the treatment groups.

Safety analyses showed a slightly lower incidence of AEs in the Custodiol-N group. Most frequently reported and with similar incidence in both treatment groups was atrial fibrillation. Drug-related or serious AEs were infrequently reported. One patient in the Custodiol-N group and 2 patients in the Custodiol® group died. None of the SAEs or deaths in this study was reported to be related to the study medication. Evaluation of laboratory parameters showed changes which can be expected following cardiac surgery and cardiopulmonary bypass; no relevant differences between the treatment groups were observed.

In conclusion, non-inferiority of Custodiol-N compared with Custodiol® with respect to their cardioprotective effect in patients undergoing cardiopulmonary bypass for coronary artery bypass surgery, was statistically confirmed based on CK-MB $AUC_{4\text{-}24h}$ as marker for myocardial damage. The safety analysis showed comparable results for both drugs.

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