TABLE 1.1
PATIENT DEMOGRAPHICS

Patient Number	Patient Initials	Age (years) at screening	Post-Dialysis Weight (kg) at screening	Sex	Dialysis Session Duration (hours)
01		30		F	
02		51		F	
03		53		F	
04		32		M	
05		66		M	
06		53		M	
07		28		F	
08		61		M	
09		59		F	
10		26		M	
11		26		M	
12		54		F	
13		55		M	
Mean		46	60.5		
SD		15	10.8		
Max		66	77.5		
Min		26	41.1		

*Patient 04 was withdrawn during Period II

*Dialysis session durations refer to the first, second and third dialysis sessions of the week, respectively

Formulation, Dosage, and Administration:

Carnitor Injection, as a sterile aqueous solution of 1 gram LC/5mL ampoule, was provided by sigma-tau (lot 916). The dose was 20 mg/kg IV after each HD session.

Sample Collection:

Study period 1 - Baseline (after second HD session of week 1):

2 mL blood samples were collected from the arterial line 5 minutes before the onset of HD, immediately after the HD had stopped (pre-dose sample), and 5, 10, 15, 30, 45 minutes, 1, 2, 4, 6, 8, 20, and 44 hours after saline administration.. The 44 hour blood sample was collected immediately before the onset of the third HD session of the week.

During the third HD session of week1, 2mL blood samples were collected simultaneously (within 30 seconds of each other) from the arterial and venous line of the dialyser at 0.25, 0.5, 1, 2, and 3 hours. At the same time, 10mL of dialysate were collected from the out-flow of the dialyser.

Study period 2 – Single dose of Carnitor (after second HD session of week 2):

Same sampling schedule as in period 1, but instead of saline, 20mg/kg Carnitor was administered.

Study period 3 – Multiple dose treatment, final dose, washout period:

a) Multiple Dose - Beginning from the third HD session of week 2 until the first HD session of week 10, within 5 minutes after the end of every HD session (i.e., 3 times per week), Carnitor was administered IV as 20mg/kg. At the second HD session of weeks 3 to 9, blood samples (2 mL) were collected pre- and post-dialysis. The post-dialysis sample was collected prior to Carnitor dosing.

b) Final dose – The final dose of Carnitor was asministered at the second dialysis session of week 10.

Blood collection and dialysate samples followed the same schedule as periods 1 and 2.

c) Washout – at the first and second dialysis session of week 11, and the second dialysis session of weeks 12, 14, and 16, arterial blood samples (2 mL) were collected pre- and post-dialysis for the washout evaluation (patients 08 –13 only).

Assay:

[redacted]

Data Analysis:

Interdialysis interval - between the second and third dialysis sessions of the week)
Intradialysis interval – during the third dialysis session of the week

INTERDIALYSIS INTERVAL

The following parameters were calculated for LC, ALC and TC

Parameter	Definition and Method of Calculation
CMAX (µM)	The maximum observed plasma concentration during the interdialysis period
TMAX (hr)	The actual time at which CMAX occurred
AUC(0-44) (µmol.hr/L)	The area under the plasma concentration versus time curve from time zero (time of dosing) until 44 hours after dosing. The 44 hour sample was collected prior to the next hemodialysis session. The area was calculated using the linear trapezoidal method, and actual blood sampling times were used.
Baseline-corrected AUC(0-44) (µmol.hr/L)	The difference between the area AUC(0-44) value obtained after Carnitor® administration (Period II and III) and the corresponding value obtained in Period I (under baseline conditions).

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INTRADIALYSIS INTERVAL

The following parameters were calculated for LC and ALC

Parameter	Definition and Method of Calculation
k (hr^{-1})	The **elimination rate constant** of the substrate during the dialysis session, calculated using log(e)-linear regression. Values for all arterial plasma samples collected during hemodialysis (0.25, 0.5, 1, 2 and 3 hours) were used in the estimation of this parameter.
Half-life (hr)	The **half-life** of the substrate during the dialysis session, calculated as 0.693/k
Cpred (µM)	The **predicted arterial plasma concentration at the end of the dialysis session**, estimated for the purpose of defining AUC(0-end). Cpred was estimated from the following equation: $Cpred = Intercept \cdot e^{(-k \cdot duration\ of\ dialysis\ session)}$ where the Intercept and the rate constant (k) were estimated using log(e)-linear regression of the arterial plasma concentration versus time data.
AUC(0-end) (µmol.hr/L)	The **area under the plasma concentration versus time curve from the start until the end of dialysis.** The area was calculated for arterial plasma, using the linear trapezoidal method. The method required the estimation of the predicted concentration in arterial plasma at the end of dialysis (Cpred).

Parameter	Definition and Method of Calculation
CL DIAL (L/hr)	The **instantaneous plasma clearance of the substrate by the dialyser** was calculated using the following equation $$\text{CL DIAL} = \frac{\text{Dialysate flow rate} \bullet \text{dialysate conc. of substrate}}{\text{arterial concentration of substrate}}$$ The numerator of this equation is the rate of excretion of substrate into dialysate. CL DIAL was estimated at each intradialysis sample collection time.
CL A-V (L/hr)	The **instantaneous blood clearance of the substrate by hemodialysis**, based upon the arterio-venous concentration difference, was calculated from the following equation: $$\frac{\text{Blood flow rate} \bullet (\text{arterial conc of substrate} - \text{venous conc of substrate})}{\text{arterial conc of substrate}}$$ This was calculated at each intradialysis sample collection time. The calculation assumes that the arterial and venous blood flows are the same and that the relative arterial and venous concentrations of the substrate in plasma reflect those in blood.
CL A-V (AUC) (L/hr)	The **time-averaged blood clearance of substrate by hemodialysis** was calculated from the following equation: $$\frac{\text{Blood flow rate} \bullet (\text{arterial area under the curve} - \text{venous area under the curve})}{\text{arterial area under the curve}}$$ CL A-V (AUC) was calculated using the area-under-the-curve values between 0.25 and 3 hours only for the arterial and venous plasma concentration versus time curves. These area terms were estimated, in turn, using the trapezoidal method. The calculation assumes that the arterial and venous blood flows are the same and that the relative arterial and venous concentrations of the substrate in plasma reflect those in blood.

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Parameter	Definition and Method of Calculation
Extraction ratio	The **extraction ratio of the substrate by the dialyser** was taken to be the time-averaged blood clearance by hemodialysis relative to the blood flow rate through the dialyser: i.e. $\text{Extraction ratio by dialyser} = \frac{\text{CL A - V (AUC)}}{\text{Blood flow rate through dialyser}}$
Volume of distribution (L)	The **volume of distribution of the substrate, referenced to arterial plasma**, was estimated from the following equation $\text{Volume of distribution} = \frac{\textit{mean}\ \text{CL DIAL}}{k}$ where the CL DIAL value used in this calculation was the arithmetic mean of the instantaneous values estimated for each intradialysis sampling time.
Amount eliminated (recovered) (µmol)	The total amount of substrate eliminated from the body by the dialyser, during an entire dialysis session, was calculated from the following equation $\text{Amount eliminated} = \text{AUC(0 - end)} \bullet \textit{mean}\ \text{CL DIAL}$ where AUC(0-end) was the value derived for arterial plasma, and the CL DIAL value was the arithmetic mean of the instantaneous values estimated for each intradialysis sampling time.
Fraction of dose recovered	The fraction of the dose of LC removed from the body, in the form of the selected substrate, during a single hemodialysis session, was taken to be the 'Amount eliminated' divided by the dose of LC administered. All values were in molar equivalents.

Results:

TABLE 3.1
L-CARNITINE PHARMACOKINETIC PARAMETERS

	ARITHMETIC MEANS ± SD		
	WEEK 1 (BASELINE)	WEEK 2 (SINGLE DOSE)	WEEK 10 (FINAL DOSE)
	INTERDIALYSIS		
AUC (0-44) (µmol.hr/L)	703.20 ± 189.58	6592.49 ± 1177.90	12093.41 ± 2501.60
Baseline Corrected AUC (0-44) (µmol.hr/L)		5889.29 ± 1141.59	11390.22 ± 2451.93
CMAX (µM)	18.98 ± 5.91	1138.56 ± 240.21	1190.26 ± 269.59
TMAX (hr:min)	40:24 ± 9:29	0:06 ± 0:03	0:06 ± 0:03
	INTRADIALYSIS		
AUC (0-end) (µmol.hr/L)	32.88 ± 9.19	104.92 ± 33.86	296.93 ± 89.38
CL DIAL (L/hr)	7.77 ± 1.69	7.51 ± 1.96	8.14 ± 1.80
CL A-V (L/hr)	13.30 ± 0.94	13.39 ± 1.70	13.40 ± 1.48
CL A-V (AUC) (L/hr)	13.44 ± 1.01	13.36 ± 1.67	13.47 ± 1.34
Extraction Ratio for Dialyser	0.7354 ± 0.0688	0.7292 ± 0.0771	0.7349 ± 0.0824
Half-Life (hr)	2.34 ± 0.69	1.76 ± 0.47	1.98 ± 0.79
Volume of Distribution (L)	25.74 ± 7.73	18.70 ± 5.43	22.78 ± 9.04
Amount Eliminated (µmol)	250.01 ± 75.74	758.09 ± 288.15	2389.91 ± 856.08
LC Dose (µmol)		7527 ± 1400	7527 ± 1400
Fraction of Dose Recovered		0.100 ± 0.028	0.318 ± 0.097

TABLE 3.2
ACETYL-L-CARNITINE PHARMACOKINETIC PARAMETERS

	ARITHMETIC MEANS ± SD		
	WEEK 1 (BASELINE)	WEEK 2 (SINGLE DOSE)	WEEK 10 (FINAL DOSE)
	INTERDIALYSIS		
AUC (0-44) (µmol.hr/L)	240.92 ± 63.11	1264.78 ± 311.96	3234.09 ± 819.64
Baseline Corrected AUC (0-44) (µmol.hr/L)		1023.86 ± 313.33	2993.17 ± 809.93
CMAX (µM)	6.98 ± 2.36	35.98 ± 11.27	90.26 ± 22.29
TMAX (hr:min)	40:24 ± 9:29	19:00 ± 12:59	29:06 ± 13:46
	INTRADIALYSIS		
AUC (0-end) (µmol.hr/L)	12.59 ± 4.51	52.07 ± 28.72	131.92 ± 45.05
CL DIAL (L/hr)	8.21 ± 2.70	8.89 ± 2.95	9.21 ± 1.75
CL A-V (L/hr)	12.65 ± 1.86	12.86 ± 1.44	12.44 ± 1.40
CL A-V (AUC) (L/hr)	13.03 ± 2.18	12.66 ± 1.51	12.48 ± 1.28
Extraction Ratio for Dialyser	0.7105 ± 0.1123	0.6940 ± 0.0983	0.6830 ± 0.0978
Half-Life (hr)	2.70 ± 1.78	2.07 ± 0.56	2.04 ± 0.62
Volume of Distribution (L)	29.46 ± 17.90	26.52 ± 10.46	26.67 ± 8.91
Amount Eliminated (µmol)	94.99 ± 21.39	423.81 ± 194.83	1209.78 ± 473.18
LC Dose (µmol)		7527 ± 1400	7527 ± 1400
Fraction of Dose Recovered		0.058 ± 0.028	0.161 ± 0.053

TABLE 3.3
TOTAL L-CARNITINE PHARMACOKINETIC PARAMETERS

	ARITHMETIC MEANS ± SD		
	WEEK 1 (BASELINE)	WEEK 2 (SINGLE DOSE)	WEEK 10 (FINAL DOSE)
	INTERDIALYSIS		
AUC (0-44) (μmol.hr/L)	1433.30 ± 420.81	8405.97 ± 1397.46	16444.00 ± 3697.19
Baseline Corrected AUC (0-44) (μmol.hr/L)		6972.67 ± 1338.50	15010.70 ± 3627.39
CMAX (μM)	40.42 ± 12.49	1234.76 ± 232.67	1232.90 ± 222.69
TMAX (hr:min)	40:24 ± 9:29	0:06 ± 0:03	0:05 ± 0:00

1.10 SUMMARY

Collectively, the results of the study yield a number of important findings:

1. The removal of LC and ALC by hemodialysis is extremely efficient and the hemodialysis procedure causes a substantial decrease in the plasma concentrations of LC and ALC. Once dialysis is complete, in patients not receiving LC supplementation, the plasma levels of LC and ALC are restored by what is likely to include movement of the compound out of slowly-equilibrating tissue stores.
2. Supplementation with LC, administered post-dialysis, does not alter the efficiency of extraction of LC and ALC by hemodialysis.
3. The administration of Carnitor® at a dose of 20 $mg.kg^{-1}$, post-dialysis, produces substantial increases in the plasma concentrations of LC and, to a lesser extent ALC, during the interdialysis period. Only a small fraction of a dose of Carnitor® is subsequently removed during the next hemodialysis session, suggesting that most of the dose moves out of the rapidly equilibrating pool and into a slowly equilibrating pool, which is likely to include muscle.

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4. The repeated administration of Carnitor®, given at a dose of 20 $mg.kg^{-1}$ after each hemodialysis session, results in accumulation of LC and ALC in plasma, reaching an apparent steady-state after about 8 weeks. At this time, about 48% of each administered dose is recovered in the subsequent hemodialysis session.

5. Upon cessation of Carnitor® administration, the pre- and post-dialysis plasma levels of LC and ALC decrease progressively, but do not return to pre-treatment levels after 6 weeks. It is suggested that during this period there is movement of LC out of a slowly equilibrating pool (probably muscle) into a pool which is in rapid equilibrium with plasma.

REVIEWER'S COMMENTS FOR STUDY ST-198-US-PK01:

1. Generally agree with results.

2. The necessity of 8 weeks of Carnitor therapy is unclear. It appears the LC concentrations and AC/LC ratio stabilize after 2 weeks of thrice weekly Carnitor therapy. Also, the sponsor indicates that steady-state is reached after 8 weeks of Carnitor therapy, based on ALC and LC concentrations. Since only single-dose and 8 week pharmacokinetic samples were obtained, it is impossible to tell if a 'steady-state was reached before the 8 weeks.

Study ST-96001 and ST-96002

Title: ST-96001

This study was a Phase 2/3, multicenter, randomized, double-blind, placebo-controlled clinical trial of four parallel groups. The patients (N=133) were divided into four treatment groups, receiving Carnitor at dose of 10, 20, or 40 mg/kg or a placebo three times a week for 24 consecutive weeks after HD.

Title: ST-96001

This study was a Phase 3, multicenter, randomized, double-blind, placebo-controlled clinical trial. The patients (N=60) were divided into two groups, receiving Carnitor 20 mg/kg or placebo three times a week for 24 consecutive weeks after HD.

The primary objectives for both studies were the improvement in VO2 max and the improvement on Quality of Life assessment. The secondary objective was to evaluate the safety and efficacy of Carnitor therapy.

Inclusion criteria were similar for both studies and included:
-over 18 years old,
-had a diagnosis of ESRD and was undergoing HD three times weekly for at least 6 months
-had a plasma AC/LC ratio more than 0.40.

Patients were excluded if they had received carnitine therapy in the 4 months prior to screening.

In both studies plasma one plasma sample was obtained from each patient at baseline, week 12, and week 24 before HD. These samples were assayed for LC, ALC, and TC.

Results for LC are included in the review; results for ALC and TC are not clinically relevant and are included here only in table format. The results indicate that concentrations of LC and TC are elevated after Carnitor therapy at 12 weeks and continue to rise at 24 weeks. For ALC, only data at baseline and 24 weeks are available and indicate that concentrations at 24 weeks are elevated after Carnitor therapy.

Protocol ST-96001

Pre-Dialysis Plasma Concentration of L-Carnitine, Total Carnitine, and Acetyl-L-Carnitine
Laboratory Test: Total Carnitine Test Units: nmol/ml

TREATMENT GROUP		VISIT Initial	Week 12	Week 24
Placebo	N	33	32	30
	MEAN	43.23	48.36	47.03
	MEDIAN	40.30	41.80	40.40
	STD	11.79	26.08	22.32
	MIN	20.10	27.40	19.30
	MAX	78.90	172.00	145.00
L-Carn 10 mg	N	34	30	28
	MEAN	41.62	184.52	223.39
	MEDIAN	37.90	176.00	209.00
	STD	15.43	102.04	71.65
	MIN	14.10	80.80	111.00
	MAX	81.60	658.00	366.00
L-Carn 20 mg	N	32	30	28
	MEAN	42.98	320.33	398.96
	MEDIAN	39.75	317.50	374.50
	STD	13.47	86.11	90.57
	MIN	26.80	160.00	231.00
	MAX	77.40	531.00	578.00
L-Carn 40 mg	N	34	30	29
	MEAN	41.00	636.20	789.83
	MEDIAN	43.45	649.50	807.00
	STD	12.75	175.48	229.19
	MIN	16.40	235.00	334.00
	MAX	66.70	1200.00	1280.00

Protocol ST-96001

Pre-Dialysis Plasma Concentration of L-Carnitine, Total Carnitine, and Acetyl-L-Carnitine

Laboratory Test: Acetyl-L-Carnitine Test Units: nmol/ml

TREATMENT GROUP		VISIT Initial	Week 12	Week 24
Placebo	N	31	0	29
	MEAN	6.55		7.64
	MEDIAN	5.74		7.73
	STD	2.82		3.43
	MIN	2.99		3.04
	MAX	13.90		18.90
L-Carn 10 mg	N	32	0	28
	MEAN	5.82		51.72
	MEDIAN	5.06		46.95
	STD	2.56		28.93
	MIN	1.87		15.90
	MAX	12.10		143.00
L-Carn 20 mg	N	30	0	28
	MEAN	6.53		113.08
	MEDIAN	5.95		105.50
	STD	2.46		47.64
	MIN	2.85		39.90
	MAX	13.90		229.00
L-Carn 40 mg	N	31	0	29
	MEAN	6.50		235.84
	MEDIAN	5.86		245.00
	STD	3.37		107.34
	MIN	1.12		23.20
	MAX	16.30		424.00

Protocol ST-96002

Pre-Dialysis Plasma Concentration of L-Carnitine, Total Carnitine, and Acetyl-L-Carnitine

Laboratory Test: Total Carnitine Test Units: nmol/ml

TREATMENT GROUP		VISIT Initial	Week 12	Week 24
Placebo	N	30	27	27
	MEAN	42.51	45.77	43.50
	MEDIAN	39.00	43.10	38.20
	STD	14.76	15.41	16.28
	MIN	23.80	15.70	20.00
	MAX	92.00	79.40	80.70
L-Carn 20 mg	N	30	25	23
	MEAN	48.81	321.56	384.30
	MEDIAN	45.95	295.00	364.00
	STD	11.05	126.80	115.55
	MIN	30.60	115.00	190.00
	MAX	74.60	644.00	709.00

Protocol ST-96002

3.3 Plasma Concentration of L-Carnitine, Total Carnitine, and Acetyl-L-Carnitine
Laboratory Test: Acetyl-L-Carnitine Test Units: nmol/ml

TREATMENT GROUP		VISIT Initial	Week 12	Week 24
Placebo	N	27	0	27
	MEAN	5.76		6.67
	MEDIAN	4.86		6.37
	STD	2.57		2.87
	MIN	2.89		2.72
	MAX	13.70		12.90
L-Carn 20 mg	N	28	0	23
	MEAN	6.03		94.70
	MEDIAN	5.39		91.80
	STD	2.26		39.67
	MIN	2.93		39.10
	MAX	11.60		197.00

REVIEWER'S COMMENTS FOR STUDY ST-96001 and ST-96002:

1. At 12 weeks of Carnitor therapy the LC concentrations are elevated even with 10 mgkg Carnitor. However, the clinical need for these extremely elevated concentrations is not known. The LC concentrations continue to rise between 12 and 24 weeks of Carnitor therapy.

Appendix 3. Assay performance

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