Statistical Analysis Plan

Multi-Center, randomized, control, phase IV trial to compare the efficacy & safety of Ursoplus® capsules (UDCA 250mg & Silymarin 140mg) versus UDCA alone versus Placebo among Compensated Chronic Liver Disease Patients

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1 Study Objective:

Compare the efficacy and safety of Ursoplus® capsules (UDCA 250mg & Silymarin 140mg) versus UDCA alone and versus Placebo among compensated chronic liver disease patients.

1.1 Primary Objective:

To assess the efficacy of Ursoplus® capsules versus UDCA 250 mg alone versus Placebo in the reduction of total serum bilirubin, Direct serum bilirubin and elevated liver Enzymes from baseline to end of the treatment (EOT) among compensated Chronic Liver Disease patients

1.2 Secondary Objectives:

- To assess the efficacy of Ursoplus® capsules, versus UDCA 250 mg alone and versus Placebo in improving the degree of steatosis measured by Vibration-controlled transient elastography with Controlled Attenuation Parameter (CAP)
- To assess the safety of Ursoplus® capsules, versus UDCA 250 mg alone and versus Placebo among compensated Chronic Liver Disease Patients who receive the treatment according to the routine clinical practice
- To describe an improvement in Quality of life (QoL), using the RAND 36-Item Health Survey. The English and Arabic RAND 36-Item Health Survey and scoring rules will be obtained.

2 STUDY DESIGN

This is a Prospective, Multi center, open-label, randomized, control, Interventional, phase IV Study will be conducted in 2 sites in Egypt; the Research Center of Air Force Specialized Hospital and Helwan University Hospital to assess the efficacy & safety of Ursoplus® capsules (UDCA 250mg & Silymarin 140mg) versus UDCA alone versus Placebo among compensated Chronic Liver Disease Patients.

This study is a clinical trial that will enroll 297 patients, based on Vibration-controlled transient elastography in screening visit; subjects will be enrolled in 2 groups:

- Group 1: with non-cirrhosis, F0, F1 and F2.
- Group 2: with advanced fibrosis and cirrhosis, F3 and F4

Subjects in each group (1 or 2) will be treated with either:

- Ursoplus® capsules (group A),
- Ursofalk® capsules (UDCA 250mg); group B (control group 1):
- Or with Placebo, in group C (control group 2), using Stratified random sampling

Generated by Data Management department (DM) of the CRO (Nagy Research) for the 3 treatment groups with a ratio of 1: 1 :1. Subjects who will be enrolled in this study will agree to the release of information and sign an informed consent. Subjects included in this study are enrolled for the collection of data that reflect the care they receive under routine clinical circumstances. All provided care should be according to protocol and the local standard of medical care.

The screening and enrollment period is planned to be 6 months. The total duration of the study/subject will be 6 months for treatment, follow-up, and End of Study visits

3 Inclusion and Exclusion Criteria:

3.1 Inclusion Criteria:

Subjects meeting all of the following criteria will be considered for enrollment in the study:

1. Male and female patients aged ≥ 18
2. Subjects with Compensated Chronic Liver Disease, defined as child 5-7.
3. Patients with mild disturbance of liver biochemical profile by one of the following criteria: (elevated Total Serum Bilirubin ≤ 3 mg/dl, or elevated Direct Serum Bilirubin ≤ 2 mg/dl, or elevated one or more of liver enzymes up to 3 times of the normal level).: (Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (AlP) & Gamma Glutamyl Transpeptidase (GGT)).
4. Non-diabetic subjects and subjects with Controlled DM-type 1 and 2 patients, HbA1C up to 7.5%
5. Non-pregnant or lactating female patients
6. Subjects who are willing to sign Informed Consent Form and ready to comply with the protocol for the duration of the study

3.2 Exclusion Criteria:

Subjects presenting with any of the following will not be included in the study:

1. Subjects with a history of hypersensitivity to any of the ingredients of the medication being studied
2. Subjects with positive PCR or positive antibodies to hepatitis C in the past 6 months
3. Subjects with positive Hepatitis B surface antigen (HBsAg)

4. Subjects with elevated liver enzymes more than 3 times of the normal level Alanine Transaminase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) & Gamma Glutamyl Transpeptidase (GGT).
5. Subjects with Primary Biliary Cirrhosis (PBC) and Primary Sclerosing Cholangitis (PSC).
6. Subjects with Child Pugh Score more than 7
7. Subjects with history of bleeding varices, as sign of decompensated liver.
8. Subjects having uncontrolled Diabetes (HBA1C above 7.5 %)
9. Subjects with any medical condition requiring the usage of medication that may interfere with the absorption, distribution, metabolism or excretion of the study drug such as the followings:
    a. Bile acid sequestering agents such as cholestyramine and colestipol,
    b. Antacids containing aluminum hydroxide.
    c. Drugs affecting lipid metabolism such as estrogens, oral and hormonal contraceptives, and clofibrate (and perhaps other lipid-lowering drugs)
10. Subjects who are receiving other liver support drugs (including drugs of the study), 1 month before study initiation.
11. Subjects with auto immune liver disease taking corticosteroid or immune suppressant
12. Pregnant or breast-feeding women
13. Use of oral contraceptives in child bearing ladies

4 Medicinal Products:

- Ursoplus® capsules (UDCA 250mg & Silymarin 140mg)
- Ursofalk® capsules (UDCA 250mg)
- Placebo

5 Endpoints:

5.1 Primary Efficacy Endpoints:

- Change in Liver Function Tests including; Total serum bilirubin, Direct Serum Bilirubin, and elevated Liver Enzymes (ALT, AST, ALP & GGT) between 3 treatment groups after 6 months of treatment (End of Study visit)

5.2 Secondary Endpoints:

- Change in degree of Steatosis from baseline (visit 1) to End of study between 3 treatment groups, measured by Vibration-controlled transient elastography with CAP.
- Improvement in Patients' quality of life (QoL) between 3 treatment groups after 3 months (visit 4) and 6 months of treatment (End of Study visit), using The RAND 36-Item Health Survey such as:
    - Physical functioning,
    - Role limitations due to physical health,
    - Role limitations due to emotional problems,
    - Energy/fatigue,
    - Emotional well-being, social functioning,
    - Pain
    - General health
- Incidence of serious/non-serious adverse events, including changes in lab tests
- AEs leading to permanent discontinuation of the study drug.

6 ASSESSMENT SCHEDULE

Subjects will be enrolled for duration of 6 months including the screening visit

- Screening visit 1 (Treatment initiation)
- Visit 2: after 1st month, follow-up 1
- Visit 3: after 2nd month, follow-up 2
- Visit 4: after 3rd month, follow-up 3
- Visit 5: after 4th month, follow-up 4
- Visit 6: after 5th month, follow-up 5

7 Determination of Sample Size:

As the primary objective is to assess the efficacy of Ursoplus® capsules (UDCA 250mg & Silymarin 140mg) versus UDCA alone versus Placebo among Egyptian NASH Patients in reduction of total serum bilirubin, and based on the previous study of [the hepatoprotective effect of Silymarin and UDCA in CHC patients][i] , which have been conducted on 40 patients; 10 patients received silymarin, 10 patients received UDCA, 10 patients received Silymarin + UDCA and 10 patients received Placebo.

And this study showed that:

The 12 weeks treatment period of Placebo did not show significant improvement regarding T Bilirubin levels 1.6 ± 1.0 Vs. 1.8 ± 0.7, *p*= 0.166

A statistically significant improvement in mean ± SD serum T bilirubin levels 1.3 ± 0.5 vs. 1.2 ± 0.5, *p* = 0.016 was obtained with 500mg/day UDCA therapy

In addition, the mean ± SD; T. bilirubin levels for the combined therapy group (Silymarin + UDCA) decreased significantly 1.4 ± 0.1 vs. 1.2 ± 0.3, p= 0.022

Given that a researcher has the null hypothesis that μ=μ0 and alternative hypothesis that μ=μ1≠ μ0, and that the population variance is known as σ2. Also, he knows that he wants to reject the null hypothesis at a significance level of α which gives a corresponding Z score, called it Zα/2. Therefore, the power function will be

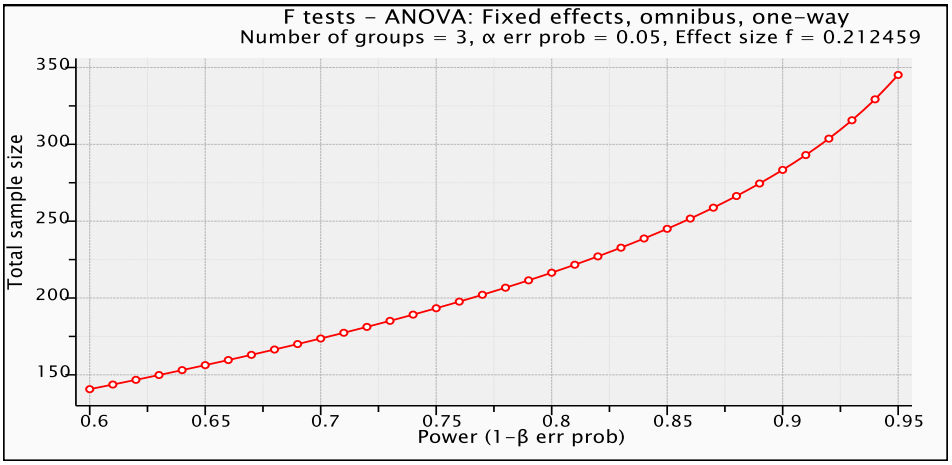
P{Z> Zα/2 or Z< -Zα/2|μ1} =1-Φ[Zα/2-(μ1-μ0)/(σ/n)]+Φ[-Zα/2-(μ1-μ0)/(σ/n)].

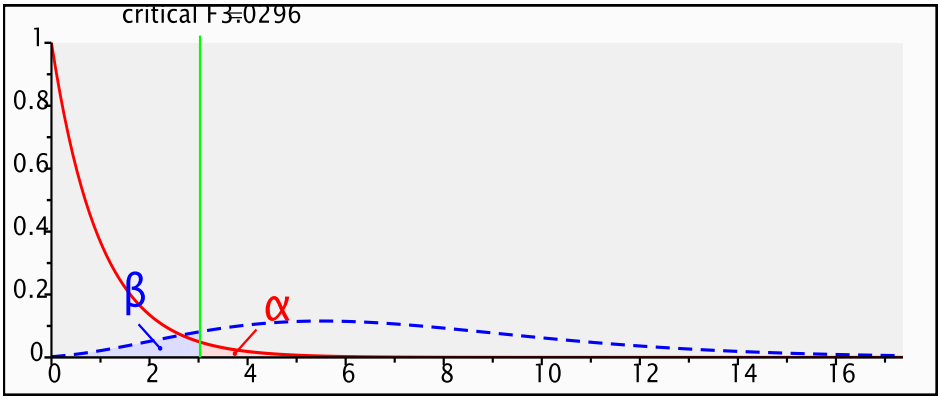
So, the following parameters are used to calculate the expected sample of the study:

Design: F tests - ANOVA: Fixed effects, omnibus, one-way, Analysis: A priori: Compute required sample size

- Input:
    - Effect size f = 0.2124591
    - α err prob = 0.05
    - Power (1-β err prob) = 0.863
    - Number of groups = 3
- Output:
    - Non-centrality parameter λ = 12.1874947
    - Critical F = 3.0295971
    - Numerator df = 2
    - Denominator df = 267
    - Total sample size = 270
    - Actual power = 0.8697572
- Confidence level 95% (α error = 5%)
- Study Power = 87%
- Sample Size = 270 Subjects (1:1:1)
- Drop out = 10% (27 subjects)
- Total number of patients: 297 subjects.
- Ursoplus Sample Size =99 subjects

- Placebo Sample Size = 99 subjects
- UDCA Sample Size = 99 subjects





8 Randomization:

Randomization is done using Stratified random sampling according to Vibration-controlled transient elastography in screening visit:

Group with non-cirrhosis (group1), F0, F1 and F2.

Group with advanced fibrosis and cirrhosis (group2), F3 and F4.

Subjects will be randomized into the 3 treatment groups with a balanced ratio of 1:1:1 by Data Management department (DM) of CRO (Nagy Research). The number of patients of the three treatment groups will be balanced (ratio 1:1:1). (99 Ursoplus®: 99 UDCA: 99 Placebo). Figure 1 shows Stratified Random Sample

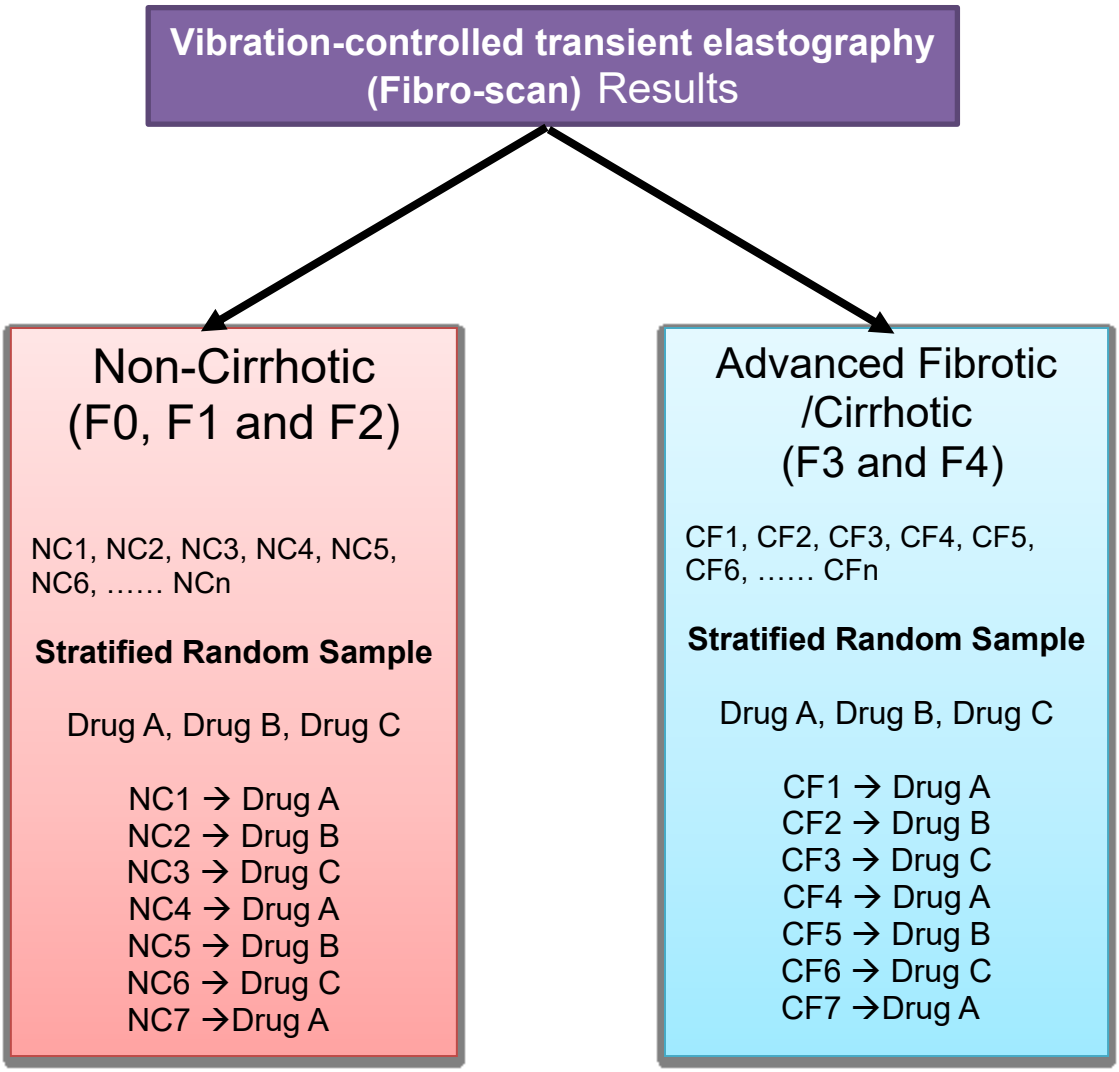


Figure 1: Stratified Random Sampling

9 Blinding

NA, as study is open label.

10 Code-Breaking:

NA

11 Data Analysis:

11.1 Test for Normality, reliability and validity:

Normality, reliability and validity tests are to be done before the main data analysis, to choose the appropriate statistics and significant tests that will be used.

11.2 Analysis populations

Analysis of all efficacy variables will be performed only for patients who completed the study without protocol violation (as per protocol).

Analysis of all safety variables will be performed for all patients (intent-to-treat population) who received even 1 dose of the treatment; either Ursoplus® capsules or UDCA or Placebo.

11.3 Statistical methods

This section provides specifications for preparation of final Statistical Analysis Plan (SAP), which will be issued prior to database lock. Any differences compared to this statistical section should be identified and documented in final SAP. Analysis will be done using SPSS version 21.

Categorical variables will be presented by number and percentage. Chi square test (or its subsidiaries) will be used to determine the p value. P -value <0.05 is considered significant.

Numerical Parametric variables will be presented by mean, SD and range. Paired t-test will be used to determine p-value between before and after treatment. Repeated measure ANOVA will be used to calculate significant effect for each group independently from baseline to end of treatment. One-way ANOVA test will be used to show and determine p-value and significance between 3 treatment groups at each point.

Numerical Non-Parametric variables will be presented by Median and interquartile range. Wilcoxon Sign Rank test will be used to determine p-value between before and after treatment. Friedman test will be used to calculate significant effect for each group independently from baseline to end of treatment. Kruskal-Wallis test will be used to show and determine p-value and significance between 3 treatment groups at each point.

11.4 Analyses variables

The following variables will be analyzed by the overall sample, comparing study treatment versus 2 controls in the 2 groups: non-Cirrhotic liver and advanced Fibroitc & Cirrhotic liver.

11.4.1 Primary analysis

A. Percent change in Liver Function Tests including; Total serum bilirubin, direct serum bilirubin and Elevated Liver Enzymes (ALT, AST, ALP & GGT) after 6 months of treatment.

11.4.2 Secondary analysis:

A. Improved degree of Steatosis from baseline (visit 1) to End of study between 3 treatment groups

B. Improved quality of life between 3 treatment groups after 3 months (visit 4) and 6 months of treatment (End of study visit), using the RAND 36-Item Health Survey such as:

- Physical functioning,
- Role limitations due to physical health,
- Role limitations due to emotional problems,
- Energy/fatigue,
- Emotional well-being, social functioning,
- Pain
- General health.

C. Serious Adverse Events (SAEs)

D. Adverse Events (AEs), including changes in any lab tests.

E. AEs leading to permanent discontinuation of the study drug.

11.5 Interim analysis

No interim report.

12 Results:

12.1 Demographic Data and Vital Signs

12.2 Primary End Point:

12.3 Secondary End Point:

12.3.1 Degree of Steatosis

12.3.2 Quality of life (RAND 36-Item Health Survey)

12.3.3 Safety Analysis

12.1 Demographic Data and vital signs

Table 1 Demographic Data and vital signs among treatment Groups

	Group 1			*p*-value	Group 2			*p*-value
	Urso	**UDCA**	**Placebo**		**Urso**	**UDCA**	**Placebo**	
Gender (male), N (%)								
Age (Yrs.), mean (SD)								
BMI, mean (SD)								
Pulse, mean (SD)								
Systolic, mean (SD)								
Diastolic, mean (SD)								

- **Medical History**

Table 2 Number and percent of medical history among treatment groups

	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
N								
Med history 1, N (%)								
Med history 2, N (%)								
Med history 3, N (%)								
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.								

12.2 Primary End Point

Table 3 Percent change of mean Total serum bilirubin at baseline and after 6 months of treatment

Total serum bilirubin	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
V1								
V6								
% Change								
p-value between V1 & V6								
p-value between 3 groups								

Table 4 Percent change of mean direct serum bilirubin at baseline and after 6 months of treatment

direct serum bilirubin	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
V1								
V6								
% Change								
p-value between V1 & V6								
p-value between 3 groups								

Table 5 Percent change of mean ALT at baseline and after 6 months of treatment

ALT	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
V1								
V6								
% Change								
p-value between V1 & V6								
p-value between 3 groups								

Table 6 Percent change of mean AST at baseline and after 6 months of treatment

AST	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
V1								
V6								
% Change								
p-value between V1 & V6								
p-value between 3 groups								

Table 7 Percent change of mean ALP at baseline and after 6 months of treatment

ALP	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
V1								
V6								
% Change								
p-value between V1 & V6								
p-value between 3 groups								

Table 8 Percent change of mean GGT at baseline and after 6 months of treatment

GGT	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
V1								
V6								
% Change								
p-value between V1 & V6								
p-value between 3 groups								

12.3 Secondary End Point

12.3.1 Degree of Steatosis

Table 9 Percent change of mean degree of Steatosis at baseline and after 6 months of treatment

Degree of Steatosis	Group 1			*p*-value	Group 2			*p*-value
	Urso	**UDCA**	**Placebo**		**Urso**	**UDCA**	**Placebo**	
V1								
V6								
% Change								
***p*-value between V1 & V6**								
***p*-value between 3 groups**								

12.3.2 Quality of life (RAND 36-Item Health Survey)

Table 10 Improved quality of life (Physical functioning, Role limitations due to physical health, Role limitations due to emotional problems, Energy/fatigue, Emotional well-being, social functioning, Pain and General health) from baseline and after 3 months of treatment

	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
V1								
V4								
% Change								
p-value between V1 & V3								
p-value between 3 groups								

Table 11 Improved quality of life (Physical functioning, Role limitations due to physical health, Role limitations due to emotional problems, Energy/fatigue, Emotional well-being, social functioning, Pain and General health) from baseline and after 6 months of treatment

	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
V1								
V6								
% Change								
p-value between V1 & V6								
p-value between 3 groups								

12.3.3 Safety Analysis

Table 12 Number and percent of Adverse Event among treatment groups

	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
Total No of AE, N (%)								
AE1, N (%)								
AE2, N (%)								
AE3, N (%)								
.								
.								

Table 13 Number and percent of Serious Adverse Event among treatment groups

	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
Total SAE, N (%)								
SAE1, N (%)								
SAE2, N (%)								
SAE3, N (%)								
.								

- **Concomitant Medication**

Table 14 Number and percent of Concomitant medication among treatment groups

	Group 1			*p*-value	Group 2			*p*-value
	Urso	UDCA	Placebo		Urso	UDCA	Placebo	
N								
ConMed1, N (%)								
ConMed2, N (%)								
ConMed3, N (%)								
.								
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[i] - SF El Menshawe, AS Ahmed, LN Abdelaty, MA Aboseif. "Study of Hepatoprotective Effect of Silymarin and Ursodeoxycholic Acid in Chronic Hepatitis C Patients". Medicine Science 2014;3(4):1655-74