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SUPPLEMENTARY INFORMATION

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Safety profile of autologous macrophage therapy for liver cirrhosis

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Domains	10^7 cells				10^8 cells				Up to10^9 cells			
	Baseline	*90 days*	*180 days*	*360 days*	*Baseline*	*90 days*	*180 days*	*360 days*	*Baseline*	*90 days*	*180 days*	*360 days*
Abdominal pain	5.33±0.00	6.00±1.20	5.56±1.02	6.00±1.00	6.56±0.77	6.33±0.67	6.11±0.84	6.78±0.39	5.11±0.51	4.55±0.39	4.67±1.20	4.33±0.67
Fatigue	5.20±0.87	7.40±1.22	5.33±2.08	5.40±1.71	5.20±2.78	5.20±2.43	4.47±2.40	5.40±1.91	2.47±0.61	3.47±0.70	3.07±0.31	2.87±0.99
Systemic symptoms	5.00±0.92	5.47±1.33	5.67±1.33	5.13±0.95	6.27±0.76	6.07±1.10	5.93±1.17	5.93±1.36	4.47±1.36	4.80±1.97	3.93±1.81	4.27±2.04
Activity	5.44±0.51	5.89±1.65	6.22±1.35	5.56±1.50	6.78±0.39	5.67±1.15	6.56±0.77	6.78±0.39	4.89±2.22	4.44±1.39	3.89±2.17	4.55±2.12
Emotional function	5.67±0.29	5.80±1.46	5.42±1.69	5.46±1.35	5.42±1.88	5.25±2.92	5.00±1.69	5.13±2.19	4.46±1.81	4.84±1.82	3.80±1.13	4.34±2.10
Worry	5.67±0.92	5.40±1.78	5.60±1.78	5.60±1.31	6.13±0.31	5.07±1.47	5.07±1.29	5.47±1.72	4.27±2.08	5.20±1.64	4.53±2.19	3.93±2.58

Supplementary Table 1. Health related quality of life scores according to Chronic Liver Disease Questionnaire (CLDQ) domains. Individual CLDQ domains are assessed using seven-point scales, ranging from the worst (1) to the best (7) possible function. Values are expressed as mean ± standard deviation, per dose group (n=3 per group) at baseline and at 90, 180 and 360 days following macrophage infusion.

Original protocol at start of phase 1 study

MAcrophage Therapy for Liver Cirrhosis

MATCH

Study Protocol

Co-sponsors	University of Edinburgh & NHS Lothian ACCORD The Queen's Medical Research Institute 47 Little France Crescent Edinburgh EH16 4TJ
Funder	Medical Research Council
Funding Reference Number	MR/M007588/1
Chief Investigator	Professor Stuart J Forbes
EudraCT Number	2015-000963-15
REC Number	15/SS/0121
ISRCTN Number	
Version Number and Date	Version 3.0 – 29 March 2016

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PROTOCOL APPROVAL

MAcrophage Therapy for Cirrhosis

MATCH

EudraCT number 2015-000963-15

MATCH 0.1

Signatures

Professor Stuart J Forbes Chief Investigator	Signature	Date
Cat Graham Trial Statistician	Signature	Date
Sponsor(s) Representative	Signature	Date
Principal Investigator	Signature	Date

LIST OF ABBREVIATIONS

ACCORD	Academic and Clinical Central Office for Research & Development - Joint office for University of Edinburgh and NHS Lothian
AE	Adverse Event
AR	Adverse Reaction
BL	Baseline
CLDQ	Chronic Liver Disease Questionnaire
CRF	Case Report Form
DLT	Dose Limiting Toxicity
DMC	Data Monitoring Committee
ELF	Enhanced Liver Fibrosis
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
ICH	International Conference on Harmonisation
IMP	Investigational Medicinal Product
ISF	Investigator Site File
LFT	Liver Function Test
MELD	Model for End Stage Liver Disease
MDMs	Mononuclear Derived Macrophages
MTD	Maximum Tolerated Dose
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SNBTS	Scottish National Blood Transfusion Service
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UAR	Unexpected Adverse Reaction
UKELD	The United Kingdom Model for End Stage Liver Disease

SUMMARY

Trial synopsis

Background

Liver disease can over time cause liver damage and scarring. Eventually it can lead to a condition called liver cirrhosis. When this happens your liver doesn't work properly and you may begin to feel unwell and tired. You can become prone to bruising, become jaundiced (yellow), and be prone to picking up infections and feel itchy. In the UK, liver disease is the fifth most common cause of death. Severe cirrhosis can sometimes be treated with a liver transplant. Unfortunately there are not enough donated livers to meet the increasing demand for transplants - and, even if there was, not everyone is suitable for a transplant.

But what if we can repair the damage?

Our research has shown that blood contains cells that can be selected and grown into cells called macrophages. In animal studies of liver disease, injections of macrophages helped to repair the liver by reducing scarring and replacing damaged liver cells.

What we want to do now

This study will test a new possible treatment for liver cirrhosis using macrophages cells grown from the patient's own blood. The first part of the study is to check the cells are safe, and find out the best number of cells to use. If the cells are safe, the second part of the study will look at whether the treatment can benefit patients with liver disease.

How we'll do it

We have developed a way to grow macrophages cells from the cirrhosis patient's blood. We take some cells from the patient's blood by using an apheresis machine which separates cells out of the blood that can be grown into macrophages. Then we test the re-injection of these cells.

What's involved for the patient

The patient will be in hospital for a few hours, looked after by highly trained nurses, and connected up to the apheresis machine by a line in the patient's vein. The cells can then be collected and stored in a bag to be taken to the laboratory to be grown, ready to be given back to the same patient a week later (again through a line into the patients vein - like having a blood transfusion).

What we hope to see

These cells may reduce the liver scarring and help the liver rebuild any damage itself. We'll get information on this by carrying out various blood and liver tests before, during and after the treatment - including a MRI scan to look at the patient's overall liver function and scarring. This will help us test if the cells can indeed be used as a treatment for people with liver disease in the future.

We also plan to find out if there are any improvements in liver fibrosis and any improvement in the quality of life for people suffering from liver disease. We also want to see if people have a better chance of getting through life without needing a liver transplant.

Objectives

The primary objectives of this study is to demonstrate safety and efficacy in this first in man study of autologous monocyte derived macrophages in participants with advanced liver cirrhosis.

Entry Criteria

Main inclusion criteria:

18≥AGE≤75

10.00≥MELD≤16.00

Aetiology, one of more of:

- Alcohol Related Liver Disease (ALD)
- Primary Biliary cirrhosis (PBC)
- Non-Alcoholic Fatty Liver Disease (NAFLD)
- Cryptogenic cirrhosis
- Haemochromotosis
- Alpha-1-Antitrypsin deficiency
- Treated (sustained viral response) hepatitis C

Diagnosis of cirrhosis – Invasive or non-invasive

Main exclusion criteria:

Decompensation

Listed for transplantation

Trial design

68-77 participants will be recruited in this single centre safety, feasibility and efficacy phase I to phase II study.

Treatment

3 participants will be recruited for validation of the GMP manufacturing process only and will not receive any macrophages (conducted under previous protocol REC ref 12/SS/0183). Assuming no adverse events, 9 participants will be recruited for the dose escalation protocol (3 participants to receive up to 10^7 cells, 3 participants up to 10^8 cells, 3 participants up to and including 10^9 cells). Given the dose escalation phase will be a 3+3 design, a maximum of 18 patients may be required if adverse events are experienced.

Outcome measures

The primary outcome measures are the safety and feasibility of re-infusion of autologous macrophages to cirrhotic participants and to establish the maximum safe dose of infusion in the phase I (dose escalation study).

Secondary outcome measures study are to demonstrate improvement in markers of liver fibrosis, improved disease related quality of life, reduced liver related clinical events and improved transplant free survival.

1. INTRODUCTION

BACKGROUND AND RATIONALE FOR STUDY

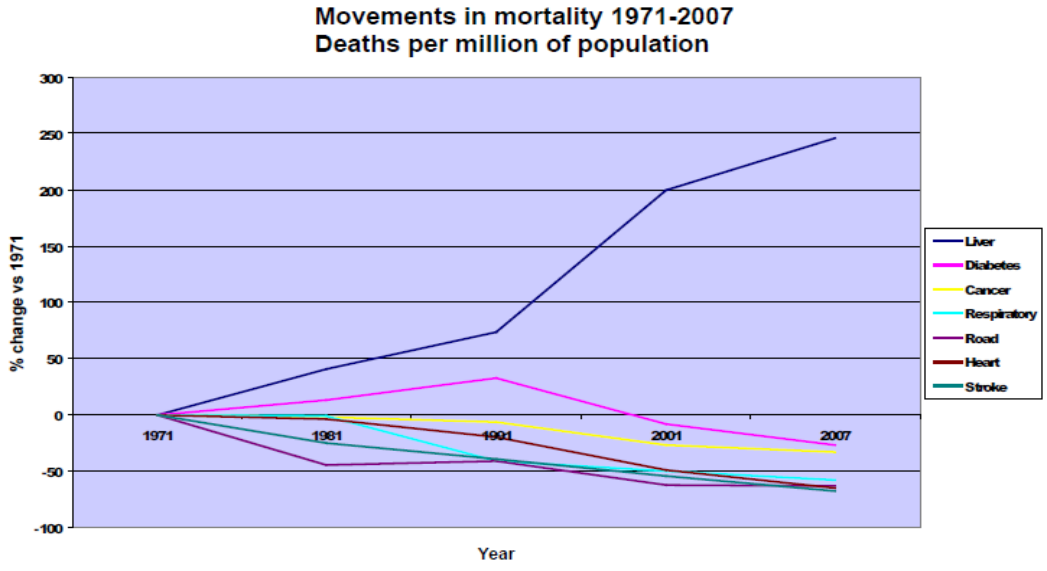
Mortality from cirrhosis in the UK has tripled over the last three decades and liver disease is the 5th commonest cause of death in the UK. 16,087 people in the UK died from liver disease in 2008, a 4.5% increase since 2007 and rates are still rapidly rising (Source: Office for National Statistics). Whilst liver transplant is curative there are insufficient suitable donor organs and many participants die waiting for a suitable organ.

Liver cirrhosis is the end result of chronic liver injury and scarring and whatever the cause (e.g. obesity, alcohol, metabolic, viral, autoimmune, cryptogenic etc.) the end result is a combination of lack of functioning liver cells and a critically increased degree of scarring. Furthermore, many participants with end-stage liver disease are not eligible for transplantation. Those who do receive transplantation require lifelong immunosuppression with the increased health risks this involves.

Autologous cell therapy has been studied and published from clinical hepatology groups in Japan, Iran, and China. The studies have typically focussed upon the use of mesenchymal stem cells (MSCs) or unpurified and heterogenous cells such as mononuclear cells which will include pro-inflammatory and pro-fibrotic cell populations[1] We have tested these cell populations in our pre-clinical models and not found them to be effective[2]).

To date there are no clinical methods of stimulating liver regeneration in the cirrhotic liver and improving liver function. There are anti-fibrotic drugs in pre-clinical testing but we are not aware of any in clinical development or practice. There are autologous cell therapies under investigation but none have shown any benefit to date in randomised trials.

Our extensive animal data has demonstrated that autologous macrophage therapy would provide a means to improve liver function by reducing fibrosis and stimulating regeneration[2-4] In cirrhotic participants a small improvement in liver function can have major benefits on both mortality and quality of life.



Source: British Liver Trust/Office for National Statistics

2. STUDY OBJECTIVES

2.1 OBJECTIVES

2.1.1 Primary Objective

Phase I (dose escalation study):

The primary objective of this study is to demonstrate safety and the maximum tolerated dose of autologous monocyte derived macrophages in participants with advanced liver cirrhosis.

Secondary Objectives

Secondary objectives are to demonstrate improvement in markers of liver fibrosis, improved disease related quality of life, reduced liver related clinical events and improved transplant free survival.

2.1.2

2.2 ENDPOINTS

2.2.1 Primary Endpoint

Phase I (dose escalation study)

The primary endpoints are the safety and feasibility and to establish the maximum safe dose of infusion of autologous macrophages to cirrhotic participants

2.2.2 Secondary Endpoints

Secondary endpoints are changes in the ELF (Enhanced Liver Fibrosis) panel, Fibroscan, Chronic Liver Disease Quality of Life, UKELD (United Kingdom Model for End Stage Liver disease), blood parameters, number of clinical events and transplant free survival.

3. STUDY DESIGN

3.1 CHOICE OF TREATMENT

Macrophages are a hetereogeneous population of cells with diverse roles within the liver including phagocytosis, maintaining immune tolerance and both promotion and resolution of inflammation and fibrosis through activation of hepatic stellate cells/production of cytokines and degradation of the extracellular matrix, respectively[5, 6].

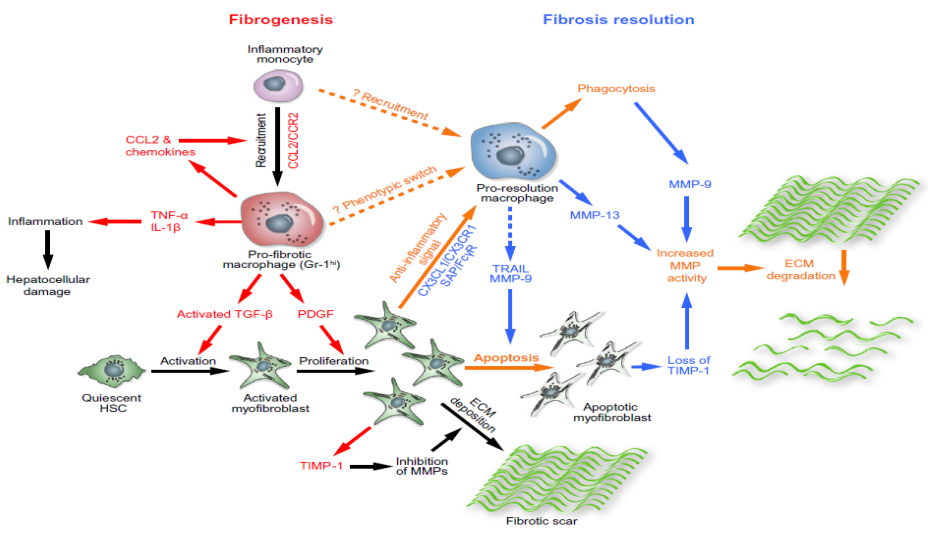


Fig. 1. Macrophages: Central regulators of hepatic fibrogenesis and fibrosis resolution.

From Ramachandran and Iredale Journal of Hepatology 2012 vol. 56 1417–1419

Previous work by our group[2] has demonstrated that administration of mature macrophages (but not undifferentiated monocytes) into a CCl_4 induced murine liver injury model results in early chemokine up-regulation leading to hepatic recruitment of endogenous macrophages, an increase in anti-inflammatory cytokines, decrease in hepatic myofibroblasts and overall improvement in clinically relevant parameters such as albumin.

We have also demonstrated the feasibility of apheresing and differentiating bone marrow derived monocytes to macrophages in cirrhotic participants.

The next stage (which will be addressed by this study) is the safety, feasibility and efficacy of infusing the macrophages back to the participant. This will be a first in human study.

3.2 JUSTIFICATION FOR DESIGN

Published studies have shown the safety of apheresis in cirrhotic participants [7-9] and current data from our group has shown the feasibility of differentiating human monocytes into macrophages from cirrhotic participants under GMP conditions[23]

This will be a phase I to II single centre study divided into 2 parts; firstly to determine the safety and maximum tolerated dose of peripherally infused macrophages to cirrhotic participants and secondly to examine the effect of the maximum tolerated dose on the severity of liver disease.

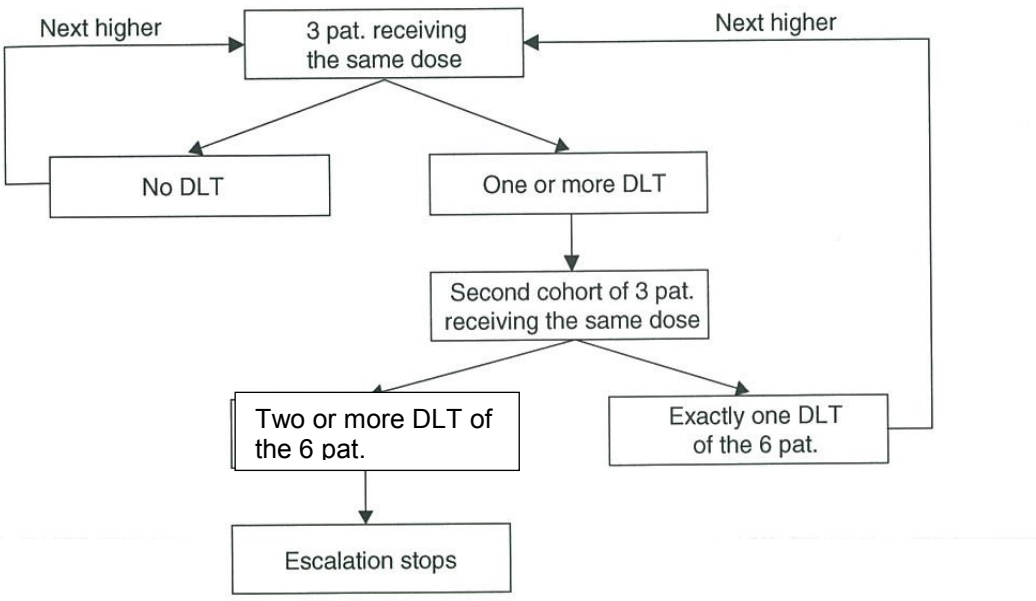
Participants will be followed up for 360 days.

Process validation:

3 participants were required to validate the GMP processing by SNBTS prior to any participants receiving a cell infusion. The format for these participants will be the same as the 'Macrophages in Regenerative Medicine in Cirrhotics' study and so conducted under this protocol (REC ref 12/SS/0183).

Dose escalation protocol:

To test cell safety and tolerability there will be a dose escalation regime starting with up to and including 10^7, up to 10^8, then up to 10^9 autologous mononuclear derived macrophages (MDMs) being infused (one infusion per participant). 3 participants will receive each dose of cells (9 participants in total assuming no dose limiting toxicity (DLT)). The dose escalation phase will be a standard 3+3 design. If an adverse event is encountered, the Data Monitoring Committee (DMC) would decide on further progression. If a serious adverse event (SAE) considered to be related to the macrophage infusion is experienced such as hypotension during cell infusion or thrombosis, no further progression will occur until DMC review. Given this design, it is possible that a maximum of 18 participants will be needed.



Flow diagram illustrating the traditional dose escalation rule *(taken from Handbook of Statistics in Clinical Oncology, Overview of Phase I Trials)*

On day 7 after apheresis to remove the mononuclear cells and following differentiation of these into macrophages under GMP conditions by the Scottish National Blood Transfusion Service (SNBTS) Cell Therapy Development Facility, the participant will return to the Clinical Research Facility in the morning. Premedication of 10mg IV Chlorphenamine, 100mg IV Hydrocortisone and 250mls normal saline will be infused 30 minutes prior to the macrophages via a cannula. The fresh MDMs will then be infused peripherally over 30 minutes followed by a further 250mls normal saline over 15-20 mins. A baseline early warning score (EWS) will be undertaken with continuous monitoring of pulse and with blood pressure checks every 5 minutes during the cell infusion and then every 15 minutes during the subsequent 2 hour observation period, then every hour for the remaining 10 hour observation period (minimum total of 12 hours observation in the CRF after cell infusion in the dose escalation phase and minimum 4 hours in the randomised controlled phase).

There will be a delay at least until day 15 between each participant until blood tests are reviewed. If a dose of 10^9 autologous cells/infusion is found to be safe and well tolerated (by the data monitoring committee (DMC), the next phase of the study assessing efficacy will then start. If it has not been possible to obtain 10^9 cells then the maximum obtained will be infused.

Dose Escalation Decision

Dose escalation requires written authorisation from:

- Data Monitoring Committee
- Trial Sponsor
- Principle Investigator

Prior to submission of data to the DMC, and during the dose escalation phase of the study, the PI must consult with an independent expert advisor (e.g clinical pharmacologist). The PI will then provide a recommendation to the DMC and sponsor.

The DMC will then review the data and provide a written summary (including the decision whether to authorise escalation or not) to the PI and Sponsor.

Following review by the Sponsor and PI, written authorisation to proceed (or reason(s) not to proceed) with dose escalation must be documented.

Correspondence relating to dose escalation should be communicated with the Sponsor via email to: resgov@accord.scot.

Dose escalation must not proceed without explicit, documented authorisation from the DMC, PI and Sponsor.

Table A: Trial Schedule Dose Escalation

	Screening	Apheresis	Cell Infusion	Safety Visit	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up	Follow-Up
	Visit 1	**Visit 2a** (within 7 days of visit 1)	**Visit 2b** (7 days after apheresis)	**Visit 2c** (Day 7)	**Visit 2d** (Day 14 days)	**Visit 3** (Day 30±3 days)	**Visit 4** (Day 60±3 days)	**Visit 5** (Day 90±3 days)	**Visit 6** (Day 180±7 days)	**Visit 7** (Day 360±7 days)
Informed consent	X									
Clinical Assessment	X	X	X	X	X	X	X	X	X	X
Vital Signs	X	X	X	X	X	X	X	X	X	X
Screening Blood Tests	X									
ECG	X									
Standard Blood Tests	X		X	X	X	X	X	X	X	X
Mandatory Microbiology	X									
Pregnancy test	X*		X*	X**		X*				X*
Abdominal USS	X								X	X
Fibroscan	X							X	X	X
ELF Panel	X					X	X	X	X	X
CLDQ	X							X	X	X
Apheresis		X								
Macrophage Infusion and observation			X							
Adverse Events	X	X	X	X	X	X	X	X	X	X
Clinical Events			X	X	X	X	X	X	X	X
Concomitant Medication	X		X	X	X	X	X	X	X	X

*women of child bearing age only, ** If test not carried out at previous visit.

4. STUDY POPULATION

4.1 NUMBER OF PARTICIPANTS

3 participants will be required for process validation and a total of 9 participants for the dose escalation safety study (3 groups of 3, assuming no adverse events. If there are adverse events, a maximum of 18 participants may be required, in accordance with the 3 +3 trial design model).

4.2 INCLUSION CRITERIA

1. Aged between 18 and 75 years (inclusive) at time of screening

2. Aetiology: one or more of:

- **Alcohol Related Liver Disease (ALD)**
  No active alcohol misuse ≥6 calendar months prior to screening. Features of chronic liver disease with a compatible history of alcohol excess (>80g/day), in the absence of other causes of chronic liver disease. Participants with other aetiologies of liver disease may drink alcohol within current recommended limits (14 units per week) for both males and females)

- **Primary Biliary Cirrhosis (PBC)**
  2 out of:

  Cholestatic LFTs
  Positive AMA (>1:40)
  Compatible Histology

  If already receiving Ursodeoxycholic Acid must be established on current dose >3 months prior to enrolment

- **Non-Alcoholic Fatty Liver Disease (NAFLD)**

  Either:

  Histological evidence of steatosis in the absence of other liver diseases

  Or:

  Imaging compatible with NAFLD (eg Fatty infiltration of liver) and one or more risk factors (e.g. elevated BMI, T2DM, Hypertriglyceridaemia, Hypertension)

  And:

  The absence of significant alcohol consumption (<20g/day) and no evidence of other causes of chronic liver disease

- **Cryptogenic cirrhosis**

  Diagnosis of cirrhosis unattributable to any other cause

- **Haemochromatosis**

  Diagnosis made on basis of compatible Biochemistry (Transferrin Sat >60%, Ferritin >400), Genotype (Homozygous C282Y or H63D, Compound Heterozygote) or Histology

- **Alpha-1 anti-trypsin deficiency**

  Diagnosis based on compatible genetic, phenotypic or histological testing.

  - **Previous Hepatitis C** (sustained viral response ie. undetectable HCV RNA 24 weeks after completion of treatment.)

3. Diagnosis of Cirrhosis – invasive or non-invasive;

Cirrhosis, defined as one of:

- Previous liver biopsy confirming histological features of cirrhosis
- Transient Elastography (Fibroscan) >15 kPa
- Clinical and radiological features that in the opinion of the investigator correlate with a diagnosis of cirrhosis

4. A MELD score of between 10.00 and 16.00 (inclusive) at time of screening

4.3 EXCLUSION CRITERIA

- Refusal or inability to give informed consent to participate in the study
- Other cause of chronic liver disease / cirrhosis not included in listed aetiologies – this is left to the clinical judgement of the investigator based on previous investigations and trial screening
- Portal Hypertensive Bleeding; active episode of bleeding requiring hospitalisation in the last 3 months where varices have not been eradicated by banding
- Ascites unless, in the opinion of the investigator, the ascites is minimal and well controlled with no increase to diuretic therapy in last 3 months
- Encephalopathy; current or requiring hospitalisation for treatment in last 3 months
- Hepatocellular Carcinoma – uncertain cases to be discussed at local hepatobiliary Multidisciplinary meeting, dysplastic or indeterminate nodules to be excluded, regenerative or other nodules to be included at discretion of MDM
- Previous diagnosis of Hepatocellular Carcinoma
- Previous organ Transplant or previous recipient of tissue
- Listed for Liver Transplantation
- Any situation that in the Investigators opinion may interfere with optimal study participation such as alcohol or drug abuse, domicile too distant from study site, potential non-compliance or inability to co-operate
- Presence of clinically relevant acute illness that in the opinion of the investigator might compromise the participant's safe participation in the study
- Presence or history of cancer within past 5 years with exception of adequately treated localised skin carcinoma, in situ cervical cancer or solid malignancy surgically excised in total without recurrence for 5 years
- Pregnancy or Breastfeeding
  Where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by positive hCG laboratory test or urine pregnancy test.

  If a female patient is of child bearing potential (between puberty and menopause) and is not pregnant, then a reliable method of contraception must be used in accordance with The Clinical Trials Facilitation Group (http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf).

  Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment.

The following methods of Birth control are those which may be considered as highly effective as these methods can achieve a failure rate of less than 1% per year when used consistently and correctly.

This would take the form of :
_ combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:

- oral
- intravaginal
- transdermal

progestogen-only hormonal contraception associated with inhibition of ovulation 1:

- oral
- injectable
- implantable 1

_ intrauterine device (IUD) 1
_ intrauterine hormone-releasing system (IUS) 1
_ bilateral tubal occlusion 1
_ vasectomised partner 1,2
_ sexual abstinence 3

1 Contraception methods that in the context of this guidance are considered to have low user dependency.
2 Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.
3 In the context of this guidance sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments.The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

- Allergy to steroids

- Active infection on the mandatory microbiology blood tests

- Immunosuppressants eg Azathioprine

4.4 CO-ENROLMENT

Co-enrolment will only be considered with non-interventional, observational studies

5. PARTICIPANT SELECTION AND ENROLMENT

5.1 IDENTIFYING PARTICIPANTS

Potential participants will be identified by their usual direct healthcare team, namely their treating Hepatologist.

The treating physician will either introduce the potential participant to the trial team or ask permission from the potential participant for the trial team to contact them (this could be done through a dedicated invite letter or a telephone call, provided initial consent for this to occur has been obtained from the treating physician).

Written participant information will be provided and there will be an opportunity for the potential participant to ask questions.

If the potential participant agrees, a further visit will be scheduled to discuss trial participation further. This will take place greater than 24 hours later.

5.2 CONSENTING PARTICIPANTS

The Investigator (or designated co-investigator as documented on the Signature and Delegation log) will obtain written informed consent for each participant prior to

performing any study related procedure. A Participant Information Sheet will be provided to facilitate this process. The Investigator will ensure that they adequately explain the aim, anticipated benefits and potential risks of taking part in the study to the participants. The Investigator should also stress that the participant is completely free to refuse to take part or withdraw from the study at any time.

The participant will be given ample time (greater than 24 hours) to read the Participant Information Sheet and to discuss their participation with others outside of the research team. The participant will be given an opportunity to ask questions which should be answered to their satisfaction. The right of the participant to refuse to participate in the study without giving a reason will be respected.

If the participant expresses an interest in participating in the study they should be asked to sign and date the latest version of the Informed Consent Form. The Investigator (or designated representative) will then sign and date the form. A copy of the Informed Consent form will be offered to the participant, a copy should be filed in the hospital notes, and the original placed in the Investigator Site File (ISF).

Details of the informed consent discussions should be recorded in the participant's medical notes. This should include date of and information regarding the initial discussion. Throughout the study the participant should have the opportunity to ask questions and any new information that may be relevant to the participant's continued participation should be shared with them in a timely manner. On occasion it may be necessary to re-consent the participant in which case the process above should be followed and the participant's right to withdraw from the study respected.

Details of all participants approached about the trial should be anonymously recorded on the Participant Screening/Enrolment Log and, if they are recruited, with the participant's prior consent, their General Practitioner (GP) should also be informed that they are taking part in the study. A GP Letter is provided for this purpose.

With consent from participants, any leftover blood samples will be kept to be used as part of future, ethically approved research studies. Samples will not be identifiable (i.e. all personal information details will be removed) and will only be labelled by the study participant number. This will include parts of the participants DNA sample which may be stored for use in future research provided that clinical identifiers are not used.

Note, in some circumstances, although unlikely, we may be unable to give the treatment. For example, if the product manufactured fails to meet the release criteria or if the patient is unwell on the day of the planned infusion. If this situation arises the cells will be either disposed of according to local policies or used for research as per consent.

5.3 SCREENING FOR ELIGIBILITY

The screening visit should occur approximately 7 days prior to apheresis. No trial specific tests or interventions which don't form part of routine standard practice should be conducted prior to the participant providing written informed consent.

- Review with participant all information pertaining to the aims and methods of the trial, including potential risks and benefits.
- If valid informed consent is obtained, (written consent form signed and dated by participant (see section 5.2)) the following screening investigations will take place
- Participant demographics recorded and participant registered

The potential participant will be asked to read the provided information and discuss their participation with family and/or friends.

- Full medical history taken including:

  - Date of diagnosis of liver disease
  - Alcohol history (confirmed by family member if possible)
  - Current / recent (90 days) medications and illnesses
  - Current / previous liver related complications (including but not limited to ascites/SBP, encephalopathy, variceal bleeding)
  - Previous Cardiovascular events (eg. MI, CVA)
  - Concomitant meds

- Full clinical examination
- Record HR, BP
- Measure Height, Weight – calculate BMI
- Obtain 12 lead ECG
- Obtain baseline abdominal USS and Transient Elastography (if this is technically achievable)
- Perform baseline MRI scan (if the other screening tests would indicate progression in the trial)
- Perform baseline CLDQ questionnaire
- Serum HCG test in women of child bearing potential.
  Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, UNLESS they are using effective methods of contraception during dosing of study treatment (as detailed at page 23).

Obtain blood for Baseline investigations: FBC, INR, fibrinogen, U&E, LFT, AFP, Calcium, serum for ELF panel, ferritin, triglycerides, CRP, serum for cytokine panel

Obtain blood for liver screen (if no previous result available); HBsAg, HCV Ab, AMA/SMA/Ig's, Ferritin, Caeruloplasmin, α1-Antitrypsin

Obtain blood for <u>mandatory microbiology</u> –

As part of the screening procedure all participants will require mandatory testing for blood borne infectious agents as per tissue quality and safety regulations for screening of products (antibody and NAT) prior to processing and storage.

These tests must be performed and be available before Leukapheresis (though are valid for 30 days) and consist of serological testing for HBV, HCV, Human Immunodeficiency Virus (HIV), Human T-Lymphotropic Virus 1 and 2 (HTLV-1, HTLV-2), Syphilis and NAT for HIV, HCV and HBV. Additional samples will be taken for repeat testing on the day of apheresis.
Some patients who have had a sustained response after treatment for hepatitis C infection, meaning there is no evidence for HCV viraemia (ie HCV RNA negative) for at least 6 months after cessation of treatment, but remain HCV antibody positive due to the presence of antibodies which are serologically positive for Hepatitis C virus may be included in the trial.

Appropriate pre-test counselling according to national guidelines will be available and in the event of an unexpected positive result, the investigator will provide initial

counselling and referral to the appropriate specialist service.

Vein assessment for suitability of apheresis by staff at the Clinical Apheresis Unit. If peripheral access is poor, discussion will be had with participant re: whether they would consider a temporary long line for the procedure in accordance with current clinical practice at the unit. This will be withdrawn after the procedure.

5.4 INELIGIBLE AND NON-RECRUITED PARTICIPANTS

Will be thanked for their interest and returned to the treating physicians care unless events suggest another course of action.

5.4.1 Withdrawal of Study Participants

Participants are free to withdraw from the study at any stage and may be withdrawn by the Investigator at any stage.

The following are justifiable reasons for the Investigator to withdraw a participant from study:

- Unacceptable toxicity
- Unforeseen events: any event which in the judgement of the Investigator makes further treatment inadvisable
- SAE requiring discontinuation of treatment
- Withdrawal of consent
- Serious violation of the study protocol (including persistent participant attendance failure and persistent non-compliance). The decision for this will be based on the clinical judgement of the Trial Physician in collaboration with the PI.
- Withdrawal by the Investigator for clinical reasons not related to the study

All participants will be included in the analysis unless they have withdrawn consent to remain in the study in which case participants will be included in the analysis up to the point they withdraw consent.

Withdrawal of Consent

Participants may withdraw consent at any time during a trial. The details of withdrawal should be clearly documented.

The following should be clearly documented in the medical notes:

- The date and reason the participant withdraws consent. If no reason for withdrawal is specified by the participant concerned this will also need to be documented in the medical notes. The participant should not be pressured in any way to give a reason for withdrawal if he/she does not wish to supply this information.

In the event of a participant becoming pregnant, they must be withdrawn from the study and followed up in accordance with the study protocol.

5.5 TREATMENT DETAILS

Medication preparation

To determine the maximum tolerated dose (MTD), participants will receive 1 infusion of macrophages 7 days post apheresis (day 1 for trial scheduling) according to standard protocol.

If participants are randomised to the treatment arm in the randomised controlled part of the study after the MTD has been determined, participants will receive 3 infusions in total (day 1, approximately day 30, approximately day 60).

The leukapheresis product will be collected under the terms of the Tissue Quality and Safety Regulations (Requirements of Statutory Instrument 2007. No 1523 enacting the requirements of the Tissue Directive (2004/23) and associated Commission Directives) at the SNBTS Clinical Apheresis Unit. CD14+ monocytes will be isolated and the macrophage cell product will be manufactured in compliance with GMP regulations under the terms of the SNBTS MIA (IMP) licence at the SNBTS Cell Therapy Facility.

To ensure viability of the re-infused cells, the cells will be delivered back to the patient via a free flowing line and not through a pump. Each patient will be monitored closely during the infusion to ensure the average flow rate prescribed is achieved.

5.6 DOSING REGIME

Dose escalation protocol: See also flow chart in section 3.2. The first three participants will receive up to and including $1x10^7$ cells. If 1 or more participants out of 3 has a 'Dose Limiting Toxicity' (DLT) then 3 additional participants will be treated at the same dose. If 2 or more patients out of the 6 get a DLT, escalation will stop. The stop/go point will be determined via the data monitoring committee (DMC) after review of this and the days 7 and 14 safety blood tests. This pattern is repeated for up to $1x10^8$ and up to $1x10^9$ cells. This will allow the maximum tolerated dose to be determined. In the unlikely event that 2 or more participants at the lowest dose (ie up to$1x10^7$) have a DLT, this will be referred to the DMC and consideration given to infusing a log order less ie. up to $1x10^5$.

5.7 DOSE CHANGES

If possible, toxicities should be managed symptomatically. If toxicity occurs the appropriate treatment will be used to ameliorate signs and symptoms and specialist opinion sought as necessary. In the event of uncontrollable toxicity or development of some specific toxicities (detailed below), the participant may be withdrawn from study therapy immediately. The recommended management and appropriate actions in relation to some specific toxicities / adverse reactions is detailed broadly below;

Acute transfusion reaction
If during the infusion and re-infusion of macrophages patient should experience any of the symptoms or/and signs listed below, the infusion will be stopped and medical review will be arranged promptly as recommended by BCSH guidelines.

- Temperature ≥38^0C or raise of 2^0C
- Hr>100
- Fall in BP of ≥30mmHg
- Collapse
- Pain (chest or abdominal)
- Dysponea or Sat<94% in room air
- Stridor
- Mucosal swelling
- New rash
- Diathesis haemorrhagica

Any other significant changes in clinical condition or observation should discussed with medical staff without discontinuation of infusion.

Allergy (Anaphylactic and Hypersensitivity reactions)

- Acute Anaphylactic Reactions with or without Life Threatening Features should be treated as per Resuscitation Guidelines 'Emergency Treatment of Anaphylactic Reactions' January 2008.
- Other Allergic Manifestations (eg. Pruritis, rash, urticaria) should be treated acutely with Chlorphenamine, Corticosteroids and Inhaled beta-agonists as

required.

Macrophage Activation Syndrome

Any Suspicion of MAS should prompt immediate specialist opinion from haematologist +/- critical care and treated as per recommendations with high dose steroid in the first instance.

Diagnostic criteria are for hemophagocytic lymphohistiocytosis (HLH) of which MAS is thought to be a related form.

Adapted from the 2004 Criteria for the diagnosis of HLH (detailed in Deane 2010(10))

Fever
Peripheral cytopenia in 2 or more lineages

- Hb <10.0 g/dl (or 9.0 g/dl in infants <4 weeks)
- Platelets ,100x 10^9/ l
- Neutrophils <1.0x 10^9/ l

Hypertriglyceridemia or hypofibrinogenemia

- Triglycerides ≥265 mg/dl
- Fibrinogen ≤150 mg/dl

Ferritin ≥500 g/dl

These tests will be checked at screening and post infusion with the other safety blood tests as per protocol. Patients will also be specifically observed for clinical signs of a fever >38 degrees Celsius, rash, Central Nervous System irritability eg. Headache and haemorrhages such as ecchymosis, purpura and mucosal bleeding(10).

If impending MAS is suspected other than the regulatory SAE notifications, the PI should be informed, the intensive care anaesthetist and haematologist on call should be informed and a discussion had re: transfer to a critical care environment. Supportive care will be given and consideration for high dose glucocorticoids and other immunosuppression eg. Cyclosporine as per current recommendations for treatment of MAS/HLH.

An assessment of the cause and severity of the reaction should be made by the Investigator and a decision made regarding continuation of treatment. Less severe reactions can be treated symptomatically and consideration given to slowing infusion rates or administering a premedication (Chlorphenamine, Corticosteroid) prior to subsequent infusions.

Dose Limit Toxicity

Mild worsening of LFTs would be expected as part of response to cellular therapy. However ALT>3 fold from BL; BIL>3 fold from BL; Creat ≥ 1.5 fold from BL; PLT<2 fold from BL; WCC<2.0 x10^9; Hb<1.5 fold from BL and MELD score >4 point from BL should be considered toxicity. An assessment of the cause and severity of the reaction should be made by the Investigator and a decision made regarding continuation of treatment or dose reduction.

5.8 PARTICIPANT COMPLIANCE

Macrophage infusions require administration by trial staff and therefore will be directly observed. Participant refusal will be directly monitored by trial staff.

5.9 CONCOMITANT MEDICATIONS

All medication that the participant is taking at the time of enrolment will be

recorded. Any changes or new medications added during the study will be recorded.

The generic drug name, daily dose, route of administration, treatment start/stop date and indication will be recorded.

Participants will be asked to limit alcohol consumption and participants with Alcoholic Liver Disease advised to abstain completely.

Any drug, if considered necessary for the participant, is permitted at the discretion of the Investigator, with the following exception:

- The introduction during the trial of Ursodeoxycholic Acid for participants with PBC. If Ursodeoxycholic Acid is introduced during trial participation, this will be noted for analysis of the efficacy data
- The introduction of immunosuppressants eg Azathioprine

6. STUDY ASSESSMENTS

6.1 SAFETY ASSESSMENTS

The leukapheresis product will be collected under the terms of the Tissue Quality and Safety Regulations (Requirements of Statutory Instrument 2007. No 1523 enacting the requirements of the Tissue Directive (2004/23) and associated Commission Directives) at the SNBTS Clinical Apheresis Unit[1]. CD14+ monocytes will be isolated and the macrophage cell product will be manufactured in compliance with GMP regulations under the terms of the SNBTS MIA (IMP) licence at the SNBTS Cell Therapy Facility.

Blood tests and ultrasound scans will be conducted in accordance with the above schedule to ensure safety of participants.

Strict observation and stop/go policy as determined by the DMC will also be adhered to in accordance with the above to ensure safety.

6.2 STUDY ASSESSMENTS/TREATMENT SCHEDULE

MELD score

The MELD score is a scoring system for assessing the severity of chronic liver disease. MELD was initially developed to determine risk of mortality within 3 months in participants with cirrhosis undergoing Transjugular Intrahepatic Portosystemic Shunt insertion[(11)]. MELD has been validated in out participants with compensated cirrhosis and across a broad spectrum of liver disease[(12)]. It is highly accurate in predicting one week, three month and one year mortality.

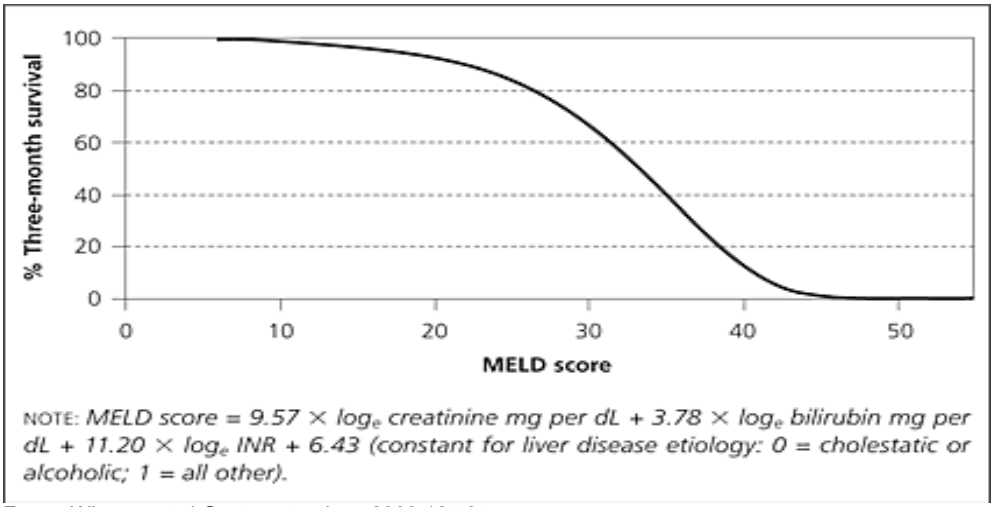
MELD independently predicts clinical decompensation in participants with compensated cirrhosis[(13)]. Further studies have shown that a change in MELD is a more significant determinant of death than initial MELD alone[(14, 15)]. Increasing MELD is also associated with the onset of ascites and encephalopathy[(16)].

For any given MELD, the magnitude and direction of change in MELD score during the previous 30 days was a significant independent mortality predictor[(15)]
Change in MELD (delta MELD) over 3 months has been shown to be useful in predicting the occurrence of variceal bleeding and encephalopathy[(17)].

[1] Some patients who have had a sustained response after treatment for hepatitis C infection, meaning there is no evidence for HCV viraemia (ie HCV RNA negative) for at least 6 months after cessation of treatment. , but remain HCV antibody positive due to the presence of antibodies which are serologically positive for Hepatitis C virus may be included in the trial

MELD score is used by all the major Western regulatory authorities involved in liver transplantation (UK Transplant, Eurotransplant and UNOS) to help prioritise the allocation of liver transplants.

The MELD score is calculated using objective variables that are readily obtained namely serum bilirubin, serum creatinine and INR.



From : Wiesner et al Gastroenterology 2003;124:94

The primary outcome measure will be change in MELD score (delta MELD) calculated using MELD at screening (Visit 1) and Day 90 MELD.

Blood samples obtained on Day 1 and Day 90 will be tested for Bilirubin, INR and Creatinine.

These results will be used to calculate MELD scores using the accepted UNOS calculation, corrected for UK units of measurement:

**MELD=10*[(0.957*ln(Creat(umol/l)*0.011312217))+(0.378*ln(Bil(umol/l)*0.05847 9
532))+(1.12*ln(INR))]+6.43[(12)]**

The following standard caveats will apply:

Any value less than one will be given a value of 1.0 once converted to US units, to avoid a negative MELD score.

If the participant has been dialysed twice in the last 7 days then the value for Creatinine will be 4.0

The maximum MELD score is 40. All values greater than 40 are given a score of 40.

The change in MELD score (delta MELD) will be calculated.

MELD (d90) – MELD (d1) = delta MELD

1.1.1
Therefore, an improvement in liver disease severity will be expressed as a negative value and deterioration in liver disease severity will be expressed as a positive value.

Blood samples obtained on Day 1 and Day 90 will be tested for Bilirubin, INR and Creatinine.

<u>31-P-MRS (MRI scan)</u>

31P-MRS will be performed at the Clinical Research Imaging Centre using our dedicated research 3T clinical imaging system (if the initial screening tests would indicate participant progression in the trial) and at day 90 post therapy. This paired imaging will be conducted for the first 10 participants randomised to the treatment arm and the first 5 randomised to control. The participant numbers are logistical rather than statistically determined. It measures the energy levels in the liver in a non-invasive and quantitative manner and reliably monitors liver regenerative activity and liver function in animal and human models[18].

Transient Elastography (Fibroscan TM, Echosens, France)

A non-invasive method for assessing liver fibrosis. Mild amplitude and low frequency vibrations (50Hz) are transmitted to the liver tissue, inducing an elastic shear wave that propagates through the underlying liver tissue. The velocity of the wave is directly related to tissue stiffness, considered as an index of the amount of fibrotic tissue. This is expressed as a numerical value in kilopascals (kPa).

It is reliable, reproducible and feasible in the majority of participants, and has high intra- and inter-observer agreement. It has been validated in most causes of chronic liver disease[19].

Participants will have a Fibroscan examination performed at screening (Visit 1), and repeated at Day 90, Day 180 and Day 360.

Enhanced Liver Fibrosis (ELF) Test

A validated panel of highly sensitive ELISA assays measuring matrix components and enzymes involved in their turnover:

Hyaluronic Acid (HA)
Tissue Inhibitor of Matrix Metalloproteinase 1 (TIMP-1)
Procollagen Type III (PIIINP)

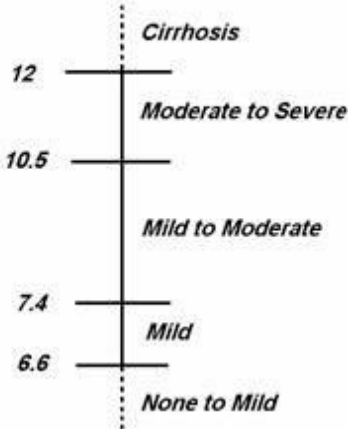
The values for each of these markers is combined in an algorithm which produces a discriminant score (the ELF score) related to the level of liver fibrosis.

The ELF score is accurate in assessing liver fibrosis in a range of chronic liver diseases and is a sensitive, specific and reproducible method for the non invasive assessment of hepatic fibrosis[20].

Participants will have blood tested and an ELF score calculated at screening (Visit 1) and at Day 30, 60, 90, 180 and Day 360.

ELF is CE marked and fully approved for research use.

Interpretation Guide :



Serum samples will be sent for analysis to

iQurLtd
UCL Institute for Liver & Digestive Health Laboratory
Room GF/701
Wolfson Lab (Ground Floor)
The Royal Free Hospital
Pond Street
London NW3 2QG
Tel: 020 7794 0500

Chronic Liver Disease Questionnaire

This is a liver specific questionnaire (Appendix 5) for measuring health related quality of life in participants with chronic liver disease[21]. It includes 29 items divided into 6 quality of life domains.

The domains are:

Abdominal symptoms
Fatigue
Systemic symptoms
Activity
Emotional function
Worry

These items are ranked on a 1 to 7 scale, providing a possible range of scores from 29 (worst quality of life) to 203 (best quality of life).

The construct validity of the CLDQ was supported by a strong correlation with participant's global rating scores. All phases of the validation process included participants with both hepatocellular and cholestatic liver disease, and disease of varying severity. It has been shown to be valid and has good test-retest reliability.

The CLDQ is self administered, takes approximately 10 minutes to complete and is designed to reflect the two weeks prior to testing.

Participants will complete a CLDQ at screening (Visit 1) and Day 90, Day 180 and Day 360. If necessary, participants can request help to complete this.

United Kingdom End Stage Liver Disease Score (UKELD)

A scoring system developed by the UK Liver Transplant Units to predict transplant waiting list mortality[22].

The score uses the parameters of Bilirubin (Bil), INR, Creatinine (Creat) and Sodium (Na) as follows:

UKELD=
[(5.395*ln(INR))+(1.485*ln(Creat)+(3.130*ln(Bil))-(81.565*ln(Na))]+435

Participants will have UKELD score calculated at screening (Visit 1) and Day 90, Day 180 and Day 360.

Individual Blood Parameters

An assessment will be made of liver related blood parameters and examined individually for changes not reflected or not assessed by MELD score.

Blood will be tested at various time points as per treatment schedule for Bilirubin,

Albumin, INR, Fibrinogen, Platelet count, White Cell Count, Haemoglobin, CRP, Triglycerides, Ferritin, ALT, AST, ALP, GGT, U&E, Calcium and AFP at screening (Visit 1) and:

Dose escalation group: Day 0, day 7, day 14, day 30, day, 60, day 90, day 180 and day 360

Control group: Day0, day 30, day 60, day 90, day 180 and day 360.

Maximum tolerated dose group: Day 0, day7, day 14, day 30, day 35 (7days after infusion), day 45 (14 days after infusion), day 60, day 65 7 days after infusion), day 75 (14 days after infusion), day 90, day 180 and day 360

Individual Blood Parameters will be measured in a CPA accredited laboratory.

Clinical Events and Transplant Free Survival

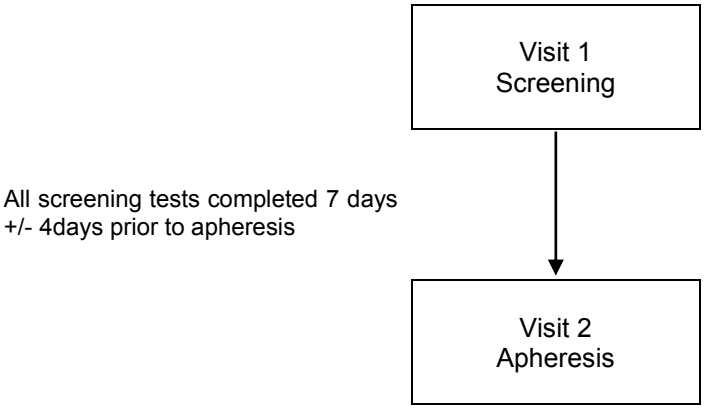
Patients will be followed for 12 months for survival. Liver related clinical events will be recorded at all scheduled visits (and any unscheduled visits) during this 12-month period according to the following criteria:

Ascites	New development of clinically significant ascites (confirmed radiologically) Worsening of established ascites (confirmed radiologically)
Encephalopathy hospitalisation	Requiring introduction of treatment or
Portal Hypertensive Bleeding	Confirmed at endoscopic examination
Spontaneous Bacterial Peritonitis	PMN cell count >250 mm3 in ascitic fluid
Hepato-Renal Syndrome	As per current diagnostic criteria
Listing for Transplantation/ Liver Transplantation	Record date and indication
Liver Cancer / Dysplastic Nodules	Record date of diagnosis
Death	Record date and cause of death

Transplant free survival is defined as the interval between the date of treatment and the date of transplant or death from any cause. Surviving transplant free patients will be censored at date last seen alive.

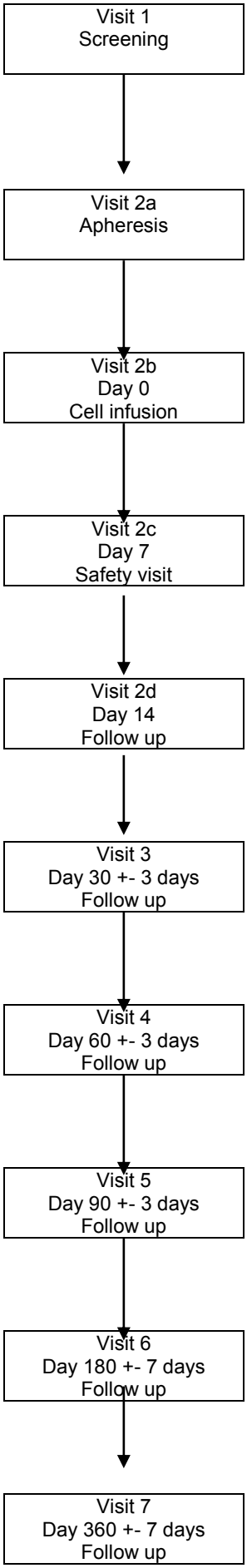
6.3 Treatment schedule

Process Validation (as per previous protocol REC ref 12/SS/0183)



Dose escalation

All screening tests completed approximately 7 +/- 4 days prior to apheresis



Treatment schedule (dose escalation)

Visit 1 (Screening as per section 5)

Visit 2a Apheresis

Visit 2b (7 days after apheresis):

- Clinical Assessment

  Inquire regarding clinical events, particularly indicators of active infection
  Inquire regarding adverse events
  Inquire regarding concomitant medications
  Perform clinical examination

- Measure HR, BP, Weight, Temp

  Pre infusion, Check FBC, CRP.

- Macrophages as per dosing schedule re-infused via peripheral vein according to standard protocol as long as temp <37.5, WCC <11, CRP <10 and no concerns re active infection. If these results are abnormal, or there are any concerns regarding the patient, advice should be sought from PI/Research Physician before administering the macrophages. The decision to carry out the reinfusion will be at discretion of the PI/Research Physician. The patients for the dose escalation phase will be in the Clinical Research Facility a minimum of 12 hours after cell infusion. There will be continuous blood pressure, pulse, oxygen saturation measurement during cell infusion, then the nurse will be physically present with the patient for the next 2 hours with the Early Warning Score measured every 15 minutes.

  FBC, U&E, Fibrinogen, INR, LFT, Ferritin, Triglycerides will be taken after infusion prior to discharge but patients do not need to wait for results if they are otherwise well

  - Perform Urinary Pregnancy Test in women of child bearing potential, **this must be completed every 28 days whilst undergoing study treatments**

- Schedule Visit 2c, (7 days after infusion) from visit 2b.

Visit 2c (7 days after infusion)

- Clinical Assessment

  Inquire regarding clinical events, particularly indicators of active infection
  Inquire regarding adverse events
  Inquire regarding concomitant medications
  Perform clinical examination

- Measure HR, BP, Weight, Temp

- Check FBC, CRP, UE, LFT, INR, Fibrinogen, Triglycerides

- Perform Urinary Pregnancy Test in women of child bearing potential, if it was not carried out in visit **2b**.

- Schedule Visit 2d (day 14) from visit 2b.

Visit 2d (14 days after infusion)

- Clinical Assessment

Inquire regarding clinical events
Inquire regarding adverse events
Inquire regarding concomitant medications
Perform clinical examination

- Measure HR, BP, Weight, Temp
- Obtain blood for FBC, INR, UE, LFT, Fibrinogen, Triglycerides, Ferritin
- Schedule Visit 3 for day 30 +/- 3 days from visit 2b

Visit 3 (Day 30)

- Clinical Assessment

  Inquire regarding clinical events
  Inquire regarding adverse events
  Inquire regarding concomitant medications
  Perform clinical examination

- Measure HR, BP, Weight, Temp

  Obtain blood for FBC, INR, UE, LFT, ELF panel

  - Perform Urinary Pregnancy Test in women of child bearing potential, **this must be completed every 28 days whilst undergoing study treatments**

- Schedule Visit 4 for day 60 +/- 3 days from visit 2b

Visit 4 (Day 60)

- Clinical Assessment

  Inquire regarding clinical events
  Inquire regarding adverse events
  Inquire regarding concomitant medications
  Perform clinical examination

- Measure HR, BP, Weight, Temp
- Obtain blood for FBC, INR, UE, LFT, ELF panel
- Schedule Visit 5 for day 90 +/- 3 days from visit 2b

Visit 5 (Day 90)

- Clinical Assessment

  Inquire regarding clinical events
  Inquire regarding adverse events
  Inquire regarding concomitant medications
  Perform clinical examination

- Perform Fibroscan
- CLDQ questionnaire

- Measure HR, BP, Weight, Temp
- Obtain blood for FBC, INR, UE, LFT, ELF panel, cytokine panel
- Schedule Visit 6 for day 180 +/- 7 days from visit 2b

Visit 6 (Day 180)

- Clinical Assessment

  Inquire regarding clinical events
  Inquire regarding adverse events
  Inquire regarding concomitant medications
  Perform clinical examination

- Perform Fibroscan
- Perform abdominal USS
- CLDQ questionnaire
- Measure HR, BP, Weight, Temp
- Obtain blood for FBC, INR, UE, LFT, ELF panel
- Schedule Visit 7 for day 360 +/- 7 days from visit 2b

Visit 7 (Day 360)

- Clinical Assessment

  Inquire regarding clinical events
  Inquire regarding adverse events
  Inquire regarding concomitant medications
  Perform clinical examination

- Perform Fibroscan
- Perform abdominal USS
- CLDQ questionnaire
- Measure HR, BP, Weight, Temp
- Obtain blood for FBC, INR, UE, LFT, ELF panel

  Perform Urinary Pregnancy Test in women of child bearing potential

7. DATA COLLECTION

The Case Report Form (CRF) will comprise the following forms:

Form	**Summary of data recorded**
Eligibility checklist	Check of Inclusion and Exclusion Criteria
Registration	Participant Demographics, Details of treatment group
Initial Assessment	Relevant Examination findings, Vital Signs, Baseline Results

Treatment Details	Dates and Dosages of Administered Treatments
90 Day Assessment	Relevant Examination Findings, Vital Signs, Day 90 Results
Follow up Assessments	Relevant Examination findings, Vital Signs, Outcomes
Concomitant Medication	Medications at Start of Study, Changes during Study
Clinical Events	Record of Events – Dates, Severity, Management and Outcomes
Adverse Effects	Record of Adverse Effects – Dates, Severity, Management and Outcomes
Adverse Event	Record of Adverse Events – Dates, Severity, Management and Outcomes

Ad hoc forms

Serious Adverse Event Form

The CRF will be completed by the Investigator or an authorised member of the research team (as delegated on the Site Signature and Delegation Log). The exception is the SAE Form which must be signed by the Investigator. See Adverse Event reporting section 8 for further details.

Entries on the CRF should be made in ballpoint pen, in blue or black ink, and must be legible. Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated. If it is not obvious why a change has been made, an explanation should be written next to the change.

Data reported on each form should be consistent with the source data or the discrepancies should be explained. If information is not known, this must be clearly indicated on the form. All missing and ambiguous data will be queried. All sections are to be completed before returning.

In all cases it remains the responsibility of the Investigator to ensure that the CRF has been completed correctly and that the data are accurate.

Completed CRFs submitted to the Clinical Research Facility will be reviewed by the Trial Co-ordinator who will enter the data into an electronic database.

8. STATISTICS AND DATA ANALYSIS

8.1 SAMPLE SIZE CALCULATION

The initial dose escalation study will be conducted as a standard 3+3 design and therefore 9 participants will be required (assuming no AEs at each dose level) up to a maximum of 18 (given if one participant has an AE another 3 will be treated at the same dose level before progression to the next, dependent on the approval of the DMC).

8.2 PROPOSED ANALYSES

Dose Escalation:

Safety and tolerability: a descriptive analysis of the safety and tolerability data will be presented.

Changes in our secondary outcome measures [ELF panel, fibroscan, CLDQ questionnaire, transplant free survival, number of clinical events, UKELD score, blood parameters (bilirubin, albumin, ALT, prothrombin time)] over the 1 year study period will be presented graphically by dose.

9. ADVERSE EVENTS

The Investigator is responsible for the detection and documentation of events meeting the criteria and definitions detailed below.

Full details of contraindications and side effects that have been reported following administration of the IMP can be found in the relevant Investigator's Brochure (IB).

Participants will be instructed to contact their Investigator at any time after consenting to join the trial if any symptoms develop. All adverse events (AE) that occur after joining the trial must be reported in detail in the Case Report Form (CRF) or AE form. In the case of an AE, the Investigator should initiate the appropriate treatment according to their medical judgement. Any AE events still present on day 360 will be confirmed and recorded as ONGOING in the Case Report Form. If appropriate, these should be handed over to the participants General Practitioner or usual care team.

9.1 DEFINITIONS

An **adverse event** (AE) is any untoward medical occurrence in a clinical trial participant which does not necessarily have a causal relationship with an investigational medicinal product (IMP).

An **adverse reaction** (AR) is any untoward and unintended response to an IMP which is related to any dose administered to that participant.

A **serious adverse event** (SAE), **serious adverse reaction** (SAR). Any AE or AR that at any dose:

- Results in death of the clinical trial participant;
- Is life threatening*;
- Requires in-participant hospitalisation^ or prolongation of existing hospitalisation;
- Results in persistent or significant disability or incapacity;
- Results in any other significant medical event not meeting the criteria above.

* Life-threatening in the definition of an SAE or SAR refers to an event where the participant was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

^ Any hospitalisation that was planned prior to randomisation will not meet SAE criteria. Any hospitalisation that is planned post randomisation will meet the SAE criteria.

A suspected unexpected serious adverse reaction (SUSAR) is any AR that is classified as serious and is suspected to be caused by the IMP, that it is not consistent with the information about the IMP in the Summary of Product Characteristics (SmPC) or Investigators Brochure.

9.2 IDENTIFYING AEs AND SAEs

All AEs will be recorded from the time a participant signs the consent form to take part in the study until completion of follow up at day 360. Any AE events still present on day 360 will be confirmed and recorded as ONGOING in the Case Report Form. If appropriate, these should be handed over to the participants General Practitioner or usual care team.

All serious adverse events (SAEs) will be reported from the date of consent until day 360.

The length of time of the SAE reporting period will remain the same for all treatment groups, irrespective of the actual therapy received. This is to ensure that the actual reporting period for SAE events is the same for each treatment group. If a participant is withdrawn from the study prior to day 90 then all SAEs will be reported until 30 days post last study infusion. Any SAE events with a start date that is the same as, or prior to the participant being withdrawn from study will be followed until resolution.

Participants will be asked about the occurrence of AEs/SAEs at every visit during the study. Open-ended and non-leading verbal questioning of the participant will be used to enquire about AE/SAE occurrence. Participants will also be asked if they have been admitted to hospital, had any accidents, used any new medicines or changed concomitant medication regimens. If there is any doubt as to whether a clinical observation is an AE, the event will be recorded.

AEs and SAEs may also be identified via information from support departments eg. Laboratories. An orange sticker detailing the study will be placed at the front of all recruited participants case notes.

9.3 RECORDING AEs AND SAEs

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (eg. Hospital notes, laboratory and diagnostic reports) related to the event. The Investigator will then record all relevant information in the CRF and on the SAE form (if the AE meets the criteria of serious).

Information to be collected includes dose, type of event, onset date, Investigator assessment of severity and causality, date of resolution as well as treatment required, investigations needed and outcome.

9.4 ASSESSMENT OF AEs AND SAEs

Seriousness, causality, severity and expectedness will be assessed by the Principal Investigator. The Investigator is responsible for assessing each AE.

The Chief Investigator (CI) may not downgrade an event that has been assessed by an Investigator as an SAE or SUSAR, but can upgrade an AE to an SAE, SAR or SUSAR if appropriate.

All Adverse Events (AEs), whether observed by staff or reported by the participant during apheresis, must be recorded including events associated with the deterioration of underlying chronic liver disease. The NCICTC AE Version 4.0 will be used to grade each AE. All initial AE grades and any change in AE grade for any particular event are to be documented. If an AE is not described in the above classification, it should be recorded as ‘other’ (refer to the CTC guide for grading).

Pre-existing conditions

A pre-existing condition must not be recorded as an AE unless the condition worsens by at least one CTC grade. This also applies to abnormal laboratory blood tests present at the time of screen ie if the blood test was abnormal at time of screen it will only be recorded as an AE if it worsens by at least one CTC grade. The condition, however, must be recorded in the CRF. All adverse events will be evaluated by the Investigator and recorded. This includes an evaluation of the seriousness and causality between the treatment and the adverse event.

All events should be graded according to the NCI CTCAE toxicity Criteria (Version 4.0).

RECORDING OF ADVERSE EVENTS

Adverse Events should be recorded on the appropriate "Treatment Form" of the CRF, including date of onset, severity, duration and relationship to study therapy and outcome.

If more than one AE occurs, each event must be recorded separately. The Investigator should take all therapeutic measures necessary for resolution of any AE. Any medication necessary for the treatment of an AE must be recorded on the concomitant medication section of the participant's CRF.

9.4.1 Assessment of Seriousness

The Investigator will make an assessment of seriousness as defined in Section 9.4

9.4.2 Assessment of Causality

The Investigator will make an assessment of whether the AE/SAE is likely to be related to the IMP according to the definitions below.

- Unrelated: where an event is not considered to be related to the IMP.

- Possibly Related: The nature of the event, the underlying medical condition, concomitant medication or temporal relationship make it possible that the AE has a causal relationship to the study drug. The assessment of causality will be made against the reference safety information found in the Investigators Brochure/Summary of Product

Where non Investigational Medicinal Products (NIMPs) eg. Rescue/escape drugs are given: if the AE is considered to be related to an interaction between the IMP and the NIMP, or where the AE might be linked to either the IMP or the NIMP but cannot be clearly attributed to either one of these, the event will be considered as an AR.

Alternative causes such as natural history of the underlying disease, other risk factors and the temporal relationship of the event to the treatment should be considered and investigated.

9.4.3 Assessment of Expectedness

If an event is judged to be an AR, the evaluation of expectedness will be made based on knowledge of the reaction and the relevant product information documented in the SmPC/IB.

The event may be classed as either:

Expected: the AR is consistent with the toxicity of the IMP listed in the SmPC/IB.

Unexpected: the AR is not consistent with the toxicity in the SmPC/IB.

9.4.4 Assessment of Severity

The Investigator will make an assessment of severity for each AE/SAE and record this on the CRF or SAE form according to one of the following

categories:

Mild: an event that is easily tolerated by the participant, causing minimal discomfort and not interfering with every day activities.

Moderate: an event that is sufficiently discomforting to interfere with normal everyday activities.

Severe: an event that prevents normal everyday activities.

Note: the term 'severe', used to describe the intensity, should not be confused with 'serious' which is a regulatory definition based on participant/event outcome or action criteria. For example, a headache may be severe but not serious, while a minor stroke is serious but may not be severe.

9.5 REPORTING OF SAEs/SARs/SUSARs

Once the Investigator becomes aware that an SAE has occurred in a study participant, the information will be reported to the ACCORD Research Governance & QA Office **immediately or within 24 hours**. If the Investigator does not have all information regarding an SAE, they should not wait for this additional information before notifying ACCORD. The SAE report form can be updated when the additional information is received.

The SAE report will provide an assessment of causality and expectedness at the time of the initial report to ACCORD according to Sections 10.4.2, Assessment of Causality and 10.4.3, Assessment of Expectedness.

The SAE form will be transmitted by fax to ACCORD on **+44 (0)131 242 9447** or may be transmitted by hand to the office or submitted via email to safety@accord.scot Only forms in a pdf format will be accepted by ACCORD via email.

Where missing information has not been sent to ACCORD after an initial report, ACCORD will contact the investigator and request the missing information.

All reports faxed to ACCORD and any follow up information will be retained by the Investigator in the Investigator Site File (ISF).

9.6 REGULATORY REPORTING REQUIREMENTS

The ACCORD Research Governance & QA Office is responsible for pharmacovigilance reporting on behalf of the co-sponsors (Edinburgh University and NHS Lothian).

The ACCORD Research Governance & QA Office has a legal responsibility to notify the regulatory competent authority and relevant ethics committee (Research Ethics Committee (REC) that approved the trial). Fatal or life threatening SUSARs will be reported no later than 7 calendar days and all other SUSARs will be reported no later than 15 calendar days after ACCORD is first aware of the reaction.

ACCORD will inform Investigators at participating sites of all SUSARs and any other arising safety information.

An Annual Safety Report/Development Safety Update Report will be submitted, by ACCORD, to the regulatory authorities and RECs listing all SARs and SUSARs.

To comply with the SNBTS' licencing agreement, all possibly related SAEs and SARs reported to ACCORD will also be reported to SNBTS by e-mail to ***NSS.SAE-SUSAR-reports@nhs.net*** within 24 hours of a report being receipted.

The sponsor will maintain a trial line listing which will be sent to SNBTS on a monthly basis

9.7 FOLLOW UP PROCEDURES

After initially recording an AE or recording an SAE, the Investigator should make every effort to follow each event until a final outcome can be recorded or reported. The Investigator should follow each AE or SAE until the event has resolved, the event is assessed as stable by the Investigator, the participant is lost to follow up, or the participant withdraws consent. Follow up information on an SAE will be reported to the ACCORD office. If, after follow up, resolution of an event cannot be established, an explanation should be recorded.

10. PREGNANCY

For female patients of child bearing age Section 4.3 of the protocol must be followed in respect of the need for contraception. .

Pregnancy is not considered an AE or SAE; however, the Investigator will collect pregnancy information for any female participants or female partners of male participants who become pregnant while participating in the study. The Investigator will record the information on a Pregnancy Notification Form and submit this to the ACCORD office within 14 days of being made aware of the pregnancy.

All pregnant female participants and partners of male participants will be followed up until following the outcome of the pregnancy.

In the event of a participant becoming pregnant, they must be withdrawn from the study and followed up in accordance with the study protocol.

11. TRIAL MANAGEMENT AND OVERSIGHT ARRANGEMENTS

11.1 TRIAL MANAGEMENT GROUP

The trial will be coordinated by a Project Management Group, consisting of the grant holders (Chief Investigator and Principal Investigator in Edinburgh), a Trial Manager and coordinating nurse.

The Trial Manager will oversee the study and will be accountable to the Chief Investigator. The Trial Manager, or an authorised member of the research team, will be responsible for checking the CRFs for completeness, plausibility and consistency. Any queries will be resolved by the Investigator or delegated member of the trial team.

A Delegation log will be prepared detailing the responsibilities of each member of staff working on the trial.

11.2 TRIAL STEERING COMMITTEE

A Trial Steering Committee (TSC) will be established to oversee the conduct and progress of the trial.

11.3 DATA MONITORING COMMITTEE

An independent Data Monitoring Committee (DMC) will be established to oversee the safety of participants in the trial. In the dose escalation phase, it will review available data at day 15 and day 45, prior to each dose escalation phase and at other intervals as determined by itself and will remain in place for the RCT phase, meeting as a minimum, every 12 months but also after the first 5 participants randomised to the cell treatment. The stop/go criteria will be determined by the DMC reviewing for example the presence of any AEs/SAEs and communicated in email/writing to the PI/research physician. This will be defined by the Common Terminology Criteria for Adverse Events (CTCAE), version 4, Grade 3, 'Severely or medically significant but not immediately life threatening: hospitalisation or prolongation of hospitalisation indicated: disabling; limiting self care ADL** This will be done after each participant in the dose escalation phase (and not after all doses)

11.4 INSPECTION OF RECORDS

Investigators and institutions involved in the study will permit trial related monitoring and audits on behalf of the sponsor, REC review, and regulatory inspection(s). In the event of an audit or monitoring, the Investigator agrees to allow the representatives of the sponsor direct access to all study records and source documentation. In the event of regulatory inspection, the Investigator agrees to allow inspectors direct access to all study records and source documentation.

11.5 RISK ASSESSMENT

An independent risk assessment will be performed by an ACCORD Clinical Trials Monitor to determine if monitoring is required and if so, at what level. An independent risk assessment will also be carried out by the ACCORD Quality Assurance Group to determine if an audit should be performed before/during/after the study and if so, at what locations and at what frequency.

11.6 STUDY MONITORING AND AUDIT

An ACCORD Clinical Trials Monitor or an appointed monitor will visit the Investigator site prior to the start of the study and during the course of the study if required, in accordance with the monitoring plan if required. Risk assessment will determine if audit, by the ACCORD QA group, is required. Details will be captured in an audit plan. Audit of Investigator sites, study management activities and study collaborative units, facilities and 3rd parties may be performed.

**Self care ADL refers to bathing, dressing and undressing, feeding self ,using the toilet, taking medications ,and not bed ridden.

12. GOOD CLINICAL PRACTICE

12.1 ETHICAL CONDUCT

The study will be conducted in accordance with the principles of the International Conference on Harmonisation Tripartite Guideline for Good Clinical Practice (ICH GCP).

A favourable ethical opinion will be obtained from the appropriate REC and local R&D approval will be obtained prior to commencement of the study.

12.2 REGULATORY COMPLIANCE

The study will not commence until a Clinical Trial Authorisation (CTA) is obtained from the appropriate Regulatory Authority. The protocol and study conduct will

comply with the Medicines for Human use (clinical Trials) Regulations 2004, as amended.

12.3 INVESTIGATOR RESPONSIBILITIES

The Investigator is responsible for the overall conduct of the study at the site and compliance with the protocol and any protocol amendments. In accordance with the principles of ICH GCP, the following areas listed in this section are also the responsibility of the Investigator. Responsibilities may be delegated to an appropriate member of study site staff.

12.3.1 Informed Consent

The Investigator is responsible for ensuring informed consent is obtained before any protocol specific procedures are carried out. The decision of a participant to participate in clinical research is voluntary and should be based on a clear understanding of what is involved.

Participants must receive adequate oral and written information – appropriate Participant Information and Informed Consent Forms will be provided. The oral explanation to the participant will be performed by the Investigator or qualified delegated person, and must cover all the elements specified in the Participant Information Sheet and Consent Form.

The participant must be given every opportunity to clarify any points they do not understand and, if necessary, ask for more information. The participant must be given sufficient time to consider the information provided. It should be emphasised that the participant may withdraw their consent to participate at any time without loss of benefits to which they otherwise would be entitled.

The participant will be informed and agree to their medical records being inspected by regulatory authorities and representatives of the sponsor9s0 but understand that their name will not be disclosed outside the hospital.

The Investigator or delegated member of the trial team and the participant will sign and date the Informed Consent Form(s) to confirm that consent has been obtained. The participant will receive a copy of this document and a copy filed in the Investigator Site File (ISF) and participant's medical notes.

12.3.2 Study Site Staff

The Investigator must be familiar with the IMP, protocol and the study requirements. It is the Investigator's responsibility to ensure that all staff assisting with the study are adequately informed about the IMP, protocol and their trial related duties

12.3.3 Data Recording

The Principle Investigator is responsible for the quality of the data recorded in the CRF. The source data plan identifies which source data correspond to CRF data and states which data are recorded directly into the CRF.

A formal letter confirming suitability for apheresis to include full history, examination findings, outcome of vein assessment conducted by trained staff and relevant blood results will be sent to the Cell Separator Unit, the Haematology Registrar covering the Cell Separator Unit and the Scottish National Blood Transfusion Service (SNBTS).

Copies of the consent forms will also be sent.

Entries on the CRF should be made in ballpoint pen, in blue or black ink,

and must be legible. Any errors should be crossed out with a single stroke, the correction inserted and the change initialled and dated. If it is not obvious why a change has been made, an explanation should be written next to the change.

12.3.4 Investigator Documentation

Prior to beginning the study, each Investigator will be asked to provide particular essential documents to the ACCORD Research Governance & QA Office, including but not limited to:

- An original signed Investigator's Declaration (as part of the Clinical Trial Agreement documents):
- Curriculum vitae (CV) signed and dated by the Investigator indicating that it is accurate and current.

The ACCORD Research Governance & QA Office will ensure all other documents required by ICH GCP are retained in a Trial Master File (TMF), where required, and that appropriate documentation is available in local ISFs.

12.3.5 GCP Training

All study staff must hold evidence of appropriate GCP training.

12.3.6 Confidentiality

All participants will be given a trial number on entry to the study. Thereafter they will be referred to by that number and by their initials on all documentation to preserve their confidentiality.

All laboratory specimens, evaluation forms, reports, and other records must be identified in a manner designed to maintain participant confidentiality. All records must be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the participant. The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study. Prior written agreement from the sponsor or its designee must be obtained for the disclosure of any said confidential information to other parties.

12.3.7 Data Protection

All Investigators and study site staff involved with this study must comply with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing and disclosure of personal information and will uphold the Act's core principles. Access to collated participant data will be restricted to those clinicians treating the participants, representatives of the sponsor(s) and representatives of regulatory authorities.

Computers used to collate the data will have limited access measures via user names and passwords.

Published results will not contain any personal data that could allow identification of individual participants.

13. STUDY CONDUCT RESPONSIBILITIES

13.1 PROTOCOL AMENDMENTS

Any changes in research activity, except those necessary to remove an apparent, immediate hazard to the participant in the case of an urgent safety measure, must be reviewed and approved by the Chief Investigator.

Amendments to the protocol must be submitted in writing to the appropriate REC, Regulatory Authority and local R&D for approval prior to participants being enrolled into an amended protocol. A log of amendments will be kept with the Product Specification File (held by SNBTS).

13.2 PROTOCOL VIOLATIONS AND DEVIATIONS

Prospective protocol deviations, i.e. Protocol waivers, will not be approved by the sponsors and therefore will not be implemented, except where necessary to eliminate an immediate hazard to study participants. If this necessitates a subsequent protocol amendment, this should be submitted to the REC, Regulatory Authority and local R&D for review and approval if appropriate.

Protocol deviations will be recorded in a protocol deviation log and logs will be submitted to the sponsors every 3 months. Each protocol violation will be reported to the sponsor within 24 hours of becoming aware of the violation.

13.3 SERIOUS BREACH REQUIREMENTS

A serious breach is a breach which is likely to effect to a significant degree:

(a) The safety or physical or mental integrity of the participants of the trial; or
(b) The scientific value of the trial.

If a potential serious breach is identified by the Chief Investigator, Principal Investigator or delegates, the co-sponsors (accord.seriousbreach@ed.ac.uk) must be notified within 24 hours. It is the responsibility of the co-sponsors to assess the impact of the breach on the scientific value of the trial, to determine whether the incident constitutes a serious breach and report to regulatory authorities and research ethics committees as necessary.

13.4 STUDY RECORD RETENTION

All study documentation will be kept for a minimum of 30 years from the protocol defined end of study point. When the minimum retention period has elapsed, study documentation will not be destroyed without permission from the sponsor.

13.5 END OF STUDY

The end of study is defined as the last participant's last visit.

The Investigators and/or the trial steering committee and/or the co-sponsor(s) have the right at any time to terminate the study for clinical or administrative reasons, as do the DMC.

The end of the study will be reported to the REC and Regulatory Authority within 90 days, or 15 days if the study is terminated prematurely. The Investigators will inform participants of the premature study closure and ensure that the appropriate follow up is arranged for all participants involved.

A summary report of the study will be provided to the REC and Regulatory Authority within 1 year of the end of the study.

13.6 INSURANCE AND INDEMNITY

The co-sponsors are responsible for ensuring proper provision has been made for insurance or indemnity to cover their liability and the liability of the Chief Investigator and staff.

The following arrangements are in place to fulfil the co-sponsors' responsibilities:

- The Protocol has been designed by the Chief Investigator and researchers employed by the University and collaborators. The University has insurance in place (which includes no-fault compensation) for negligent harm caused by poor protocol design by the Chief Investigator and researchers employed by the University. The University has insurance in place to cover any liability incurred by the sites or by SNBTS as a result of claims and proceedings (including any settlements or ex gratia payments made with the consent of the University and the site/SNBTS as applicable and reasonable legal and expert costs and expenses) made against them by or on behalf of an individual taking part in the study for personal injury (including death) to such individual arising out of or relating to any clinical intervention, treatment or procedure provided for or required by this protocol to which such individual would not have been exposed but for his or her participation in the study, where such claim or proceedings arises through the negligent act or omission of the University or as a result of those parties undertaking the study in accordance with this protocol

- Sites participating in the study will be liable for clinical negligence and other negligent harm to individuals taking part in the study and covered by the duty of care owed to them by the sites concerned. (but will not be liable where the liability arises out of or relating to any clinical intervention, treatment or procedure provided for or required by this protocol to which such individual would not have been exposed but for his or her participation in the study where the claim or proceedings arises through the negligent act or omission of the University or as a result of the sites undertaking the study in accordance with this protocol. -such liability will be covered by the insurance obtained by the University referred to above). The co-sponsors require individual sites participating in the study to arrange for their own insurance or indemnity in respect of their liabilities.

- Sites which are part of the United Kingdom's National Health Service and SNBTS will have the benefit of NHS Indemnity or in relation to the National Health Service in Scotland, the Clinical Negligence and Other Risks Indemnity Scheme

- Sites out with the United Kingdom will be responsible for arranging their own indemnity or insurance for their participation in the study, as well as for compliance with local law applicable to their participation in the study.

- The manufacturer (SNBTS) will develop GMP methodologies, secure appropriate regulatory approvals and manufacture the IMP for the study. Where it has been negligent in carrying out any of same and death or personal injury occurs to an individual taking part in the study, it will be liable. However, it will not be liable where liability arises out of or relating to any clinical intervention, treatment or procedure provided for or required by this protocol to which such individual would not have been exposed but for his or her participation in the study where such claim or proceedings arises through the negligent act or omission of the University or as a result of SNBTS undertaking the study in accordance with this protocol. Such liability will be covered by the insurance obtained by the University referred to above.

14. REPORTING, PUBLICATIONS

14.1 AUTHORSHIP POLICY

Ownership of the data arising from this study resides with the study team. On completion of the study, the study data will be analysed and tabulated, and a clinical study report will be prepared in accordance with ICH guidelines.

14.2 PUBLICATION

The clinical study report will be used for publication and presentation at scientific meetings. Investigators have the right to publish orally or in writing the results of the study.

Summaries of results will also be made available to Investigators for dissemination within their clinics (where appropriate and according to their discretion).

14.3 PEER REVIEW

Results of this trial will be submitted for publication in a peer reviewed journal.

15 INVESTIGATIONAL MEDICINAL PRODUCT

15.1.0 Study Drug Manufacturer

SNBTS Cell Therapy Development Centre,
Scottish Centre Regenerative Medicine, Little France
(MIA(IMP) 3473, MHRA site number 4883575)

15.1.1 Marketing Authorisation Holder

N/A

15.1.2 Labelling and Packaging

SNBTS Cell Therapy Development Centre,
Scottish Centre Regenerative Medicine, Little France

15.1.3 Storage

Ambient, controlled in a validated transport box at 18-24oC.

15.1.4 Summary of Product Characteristics or Investigators Brochure

The Investigators Brochure is given in appendix 1.

15.2 OVERDOSE

This will be unlikely as the cells will be prepared and then delivered in a sealed bag for infusion. Products will also be released by a QP named on the SNBTS MIA(IMP) licence according to defined SNBTS procedures in compliance with Annex 16. Release will be two-stage, with initial release based upon available BacT/ALERT sterility results and endotoxin data, then final QP release once all sterility and environmental monitoring data is available.

15.3 OTHER MEDICATIONS

15.3.1 Non-Investigational Medicinal Products

Chlorphenamine 10mg and hydrocortisone 100mg IV administered 30 minutes ahead of macrophage infusion to minimise the likelihood of allergic reaction.

15.3.2 Permitted Medications

No restrictions

15.3.3 Prohibited Medications

No restrictions other than biological or immunosuppression treatment such as anti TNF or azathioprine. If these medications need to be started on clinical grounds, continued trial participation will be at the discretion of the chief/principle investigator.

APPENDIX 1: Investigator brochure (see separate document)

APPENDIX 2

WMA DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53th WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of participants, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my participant will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the participant's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on participants or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.
17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately

stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.
26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious l participants, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.
C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE
31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the participants who serve as research subjects.
32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

- The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
- Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the participants who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, participants entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the participant which aspects of the care are related to the research. The refusal of a participant to participate in a study or the participant's decision to withdraw from the study must never interfere with the participant-physician relationship.
35. In the treatment of a participant, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the participant or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly **available.**

APPENDIX 3:

CHRONIC LIVER DISEASE QUESTIONNAIRE

Participant Number Participant TNO

Date of Questionnaire

Assessment

This questionnaire is designed to find out how you have been feeling during the last two weeks. You will be asked about your symptoms related to your liver disease, how you have been affected in doing activities, and how your mood has been.

Please complete all of the questions and select only **one** response for each question.

1. How much of the time during the last two weeks have you been troubled by a feeling of abdominal bloating?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

2. How much of the time have you been tired or fatigued during the last two weeks?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

3. How much of the time during the last 2 weeks have you experienced bodily pain?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

4. How often during the last two weeks have you felt sleepy during the day?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

5. How much of the time during the last two weeks have you experienced abdominal pain?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

6. How much of the time during the last two weeks has shortness of breath been a problem for you in your daily activities?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

7. How much of the time during the last two weeks have you not been able to eat as much as you would like?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

8. How much of the time in the last two weeks have you been bothered by having decreased strength?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

9. How often during the last two weeks have you had trouble lifting or carrying heavy objects?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

10. How often during the last two weeks have you felt anxious?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

11. How often during the last two weeks have you felt a decreased level of energy?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

12. How much of the time during the last two weeks have you felt unhappy?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

13. How often during the last two weeks have you felt drowsy?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

14. How much of the time during the last two weeks have you been bothered by a limitation of your diet?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

15. How often during the last two weeks have you been irritable?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

16. How much of the time during the last two weeks have you had difficulty sleeping at night?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

17. How much of the time during the last two weeks have you been troubled by a feeling of abdominal discomfort?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

18. How much of the time during the last two weeks have you been worried about the impact your liver disease has on your family?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

19. How much of the time during the last two weeks have you had mood swings?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

20. How much of the time during the last two weeks have you been unable to fall asleep at night?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

21. How often during the last two weeks have you had muscle cramps?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time

6 Hardly any of the time
7 None of the time

22. How much of the time during the last two weeks have you been worried that your symptoms will develop into major problems?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

23. How much of the time during the last two weeks have you had a dry mouth?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

24. How much of the time during the last two weeks have you felt depressed?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

25. How much of the time during the last two weeks have you been worried about your condition getting worse?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

26. How much of the time during the last two weeks have you had problems concentrating?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

27. How much of the time have you been troubled by itching during the last two weeks?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time

5 A little of the time
6 Hardly any of the time
7 None of the time

28. How much of the time during the last two weeks have you been worried about never feeling any better?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

29. How much of the time during the last two weeks have you been concerned about the availability of a liver if you need a liver transplant?

1 All of the time
2 Most of the time
3 A good bit of the time
4 Some of the time
5 A little of the time
6 Hardly any of the time
7 None of the time

APPENDIX 4

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