A MULTICENTRE, PHASE II, OPEN LABEL, RANDOMISED CONTROLLED TRIAL OF REPEATED AUTOLOGOUS INFUSIONS OF G-CSF MOBILISED CD133+ BONE MARROW STEM CELLS IN PATIENTS WITH CIRRHOSIS

REPEATED AUTOLOGOUS INFUSIONS OF STEM CELLS IN CIRRHOSIS

REALISTIC

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AMENDMENTS

The following amendments and/or administrative changes have been made to this protocol since the date of preparation

Amendment No.	Date of Amendment	Version No.	Type of amendment (e.g. substantial/non-substantial)
1	**21st May 2009**	**2.0**	Substantial Amendment a) Section 3.2 - Further Exclusion Criteria added b) Section 7.4, 7.5 - Addition of Treatment Day 4 Blood Test c) Section 7.9 - Lenograstim Discontinuation Criteria added
2	**5th November 2009**	**3.0**	Substantial Amendment a) Section 3.2 - Further detail added to Exclusion Criteria. b) Section 7.1 – Change to study administration (all drug will be administered by suitable qualified medical staff only). c) Section 7.2 – Dose modification and toxicity management recommendations (new section). d) Section 8.0 – Additional information added to adverse event reporting section, clarification of SAE reporting period and procedures. e) Section 13.0 Additional information added to power calculations, Interim and final analysis sections f) Section 14.1 Change in sponsor details : single sponsor to Co-sponsorship g) Appendix 2 – change to questionnaire layout. A number of minor amendments have been made throughout the protocol. Which include change in study personnel, Use of µg to replace mcg
3	**6th July 2011**	**4.0**	Substantial Amendment **a)** Section 3.1 amended inclusion MELD range **b)** Section 4 changed wording to say multi centre **c)** Section 5.3, 7.4, 7.5, 7.6: Increased ELF testing frequency A number of minor changes have been made through the protocol, which includes change in study personnel.

4	**22nd February 2012**	**5.0**	Substantial Amendment a) Changes to inclusion criteria and addition of new inclusion criteria • Alpha-1 Antitrypsin Deficiency • Changes to some diagnostic requirements relating to aetiology of the liver disease. b) Change to wording of exclusion criteria • The requirement (time scales) for Ascites, Portal hypertensive bleeding and Encephalopathy (requiring treatment or hospitalisation) free period prior to randomisation has been reduced from 6 months to 3 months. A number of minor changes have been made throughout the protocol, which includes change in study personnel.
5	**24th May 2012**	**6.0**	Substantial Amendment a) Change of sponsor details from co-sponsor to single sponsor b) Change to IMP label c) update to UKELD information
6	**7th November 2012**	**7.0**	Substantial Amendment a) Change to inclusion criteria (MELD range) b) Change to inclusion criteria (Age range) A number of minor changes have been made throughout the protocol, which includes change in study personnel and information on eRDC.
7	**5th March 2015**	**8.0**	Substantial Amendment A number of minor changes to the protocol. Section 11 : Statistical Considerations Changes to the primary statistical analysis to include MELD measurements at baseline, 30, 60 and 90 days.

CHIEF INVESTIGATOR SIGNATURE PAGE

REALISTIC TRIAL

A MULTICENTRE, PHASE II, OPEN LABEL, RANDOMISED CONTROLLED TRIAL OF REPEATED AUTOLOGOUS INFUSIONS OF G-CSF MOBILISED CD133+ BONE MARROW STEM CELLS IN PATIENTS WITH CIRRHOSIS

Version 8.0, 5th March 2015

This Protocol is approved by :

Professor Philip Newsome **Signature :** **Date :**

Chief Investigator

TRIAL SYNOPSIS

Background

Liver disease mortality and morbidity is rapidly rising and liver transplantation is limited by organ availability and associated risks.
Animal data suggests that Haematopoietic Stem Cells (HSCs) and Granulocyte Colony Stimulating Factor (G-CSF) play an important role in i) increasing hepatic regeneration and ii) reducing hepatic fibrosis.
Small scale human studies have shown that Stem Cell therapy is safe and feasible and has suggested clinical benefit.
No published studies have yet examined the effect of Stem Cell therapy in a randomised controlled trial and no studies have evaluated the effect of repeated therapy.

Objectives

The primary objective is to demonstrate an improvement in the severity of liver disease over 3 months using either G-CSF alone or G-CSF followed by repeated infusions of HSCs compared with standard conservative management. The 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.

Entry Criteria

Main Inclusion Criteria

- 18≤ AGE ≤75
- 11.00≤ MELD ≤15.50
- Aetiology, one or more of:
  - Alcohol Related Liver Disease (ALD)
  - Hepatitis C (HCV)
  - Hepatitis B (HBV)
  - Primary Biliary Cirrhosis(PBC)
  - Non-Alcoholic Fatty Liver Disease (NAFLD)
  - Cryptogenic Cirrhosis
  - Haemochromatosis
  - Alpha-1 Antitrypsin deficiency

Main Exclusion Criteria

- Diagnosis of Cirrhosis - invasive or non-invasive
- Decompensation
- Listed for transplantation

Trial Design

Eighty-one patients will be recruited into this multi centre, early phase, randomised controlled trial

Treatments

Patients will be randomised to one of three trial groups:

Group 1	Control group, Standard conservative management
Group 2	Treatment: G-CSF on Days 1-5
Group 3	Treatment: G-CSF on Days 1-5 followed by Leukapheresis, CD133+ cells isolated, aliquoted and frozen. Repeated infusions via peripheral vein on Days 5/6, 30, 60.

Outcome Measures

The primary outcome measure is the Change in MELD score (delta MELD) at 3 months from randomisation. Secondary outcome measures are ELF panel, Fibroscan, Chronic Liver Disease Quality of Life, UKELD, Blood parameters, number of Clinical events and Transplant Free Survival.

TRIAL SCHEDULE

	Screening	Treatment					Follow Up	
	Visit 1 (screening)[1,2]	Visit 2a – 2e[1] (1st – 5th Treatment Day)[7]	Visit 2f (5th/6th Treatment Day)[7]	Visit 3 (Day 30)	Visit 4 (Day 60)	Visit 5 (Day 90)	Visit 6 (Day 180)	Visit 7 (Day 360)
Informed Consent	X							
Clinical Assessment[2]	X	X	X	X	X	X	X	X
Vital Signs[3]	X	X	X	X	X	X	X	X
Screening Blood Tests[4]	X							
ECG	X							
Standard Blood Tests[5]	X	X	X	X	X	X	X	X
Mandaory Microbiology[7]	X							
Abdominal USS	X		X				X	
Fibroscan	X					X	X	X
ELF Panel	X			X	X	X	X	X
CLDQ	X					X	X	X
G-CSF Administration		GROUP 2 GROUP 3						
Leukapheresis			GROUP 3					
Blood Test for Circ CD34+		GROUP 2 GROUP 3	GROUP 2 GROUP 3					
Blood Test for Circ CD133+		GROUP 2 GROUP 3	GROUP 2 GROUP 3					
CD133+ Cell Infusion			GROUP 3[8]	GROUP 3	GROUP 3			
Adverse Effects[6]	X	X	X	X	X	X	X	X
Clinical Events[6]		X	X	X	X	X	X	X
Concomitant Medication	X	X	X	X	X	X	X	X

1. For patients in Groups 1 and 2 Visit 1 and visit 2a should be combined into one day where possible. . For patients in Group 3, timing of visit 2a will depend on scheduling of Leukapheresis.
2. All screening tests must be completed less than 7 days prior to randomisation and treatment and must start less than 7 days following randomisation. Day of randomisation will be considered as Day 1 for scheduling purposes.
2. Clinical Assessment consists of Complete History and Examination at Screening and Focussed History and Relevant Examination at subsequent Visits
3. Vital Signs to include Heart Rate, Blood Pressure, Temperature and Weight
4. Screening Blood tests as detailed in Section 5.

5. Standard Blood Tests consists of Full Blood Count, Urea and Electrolytes, Liver Function Tests, Magnesium and Alanine Aminotransferase (ALT) International Normalised Ratio (INR).
6. Adverse Effects and Clinical Events will be monitored continuously until completion of follow up. Serious Adverse Events (SAE's) will be reported from the date of consent. All adverse events experienced by patients will be recorded irrespective of the causality. (see section 7)
7. Mandatory microbiological testing must be performed between 7 and 30 days prior to Leukapheresis - HBV, HCV, Human Immunodeficiency Virus (HIV), Human T-Lymphotrophic Virus 1 and 2 (HTLV-1, HTLV-2) and Syphilis.
8. The first Reininfusion of CD133+ (group 3 patients only) will occur on one occasion only between days 6-10. The timing will be determined by the timing of each patients leukapheresis. CD133+ cell isolation and the local hospital arrangements (see section 7.6 in the study protocol)

ABBREVIATIONS

AFP	Alpha Feto Protein
ALD	Alcoholic Liver Disease
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BM	Bone Marrow
BMI	Body Mass Index
BMSC	Bone Marrow Stem Cell
BP	Blood Pressure
CLDQ	Chronic Liver Disease Questionnaire
ELF	Enhanced Liver Fibrosis test
EPC	Endothelial Progenitor Cell
FBC	Full Blood Count
G-CSF	Granulocyte Colony Stimulating Factor
GGT	Gamma Glutamyl Transferase
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HR	Heart Rate
HSC	Haematopoietic Stem Cell
INR	International Normalised Ratio
LFT	Liver Function Tests
MAPC	Multi-potent Adult Progenitor Cell
MELD	Model for End stage Liver Disease score
MSC	Mesenchymal Stem Cell
NAFLD	Non Alcoholic Fatty Liver Disease
OLT	Orthotopic Liver Transplant
PBC	Primary Biliary Cirrhosis
PBSC	Peripheral Blood mobilised Stem Cells
rHuG-CSF	Recombinant Human Granulocyte Colony Stimulating Factor
SBP	Spontaneous Bacterial Peritonitis
U&E	Urea, Creatinine and Electrolytes
UKELD	United Kingdom End stage Liver Disease score

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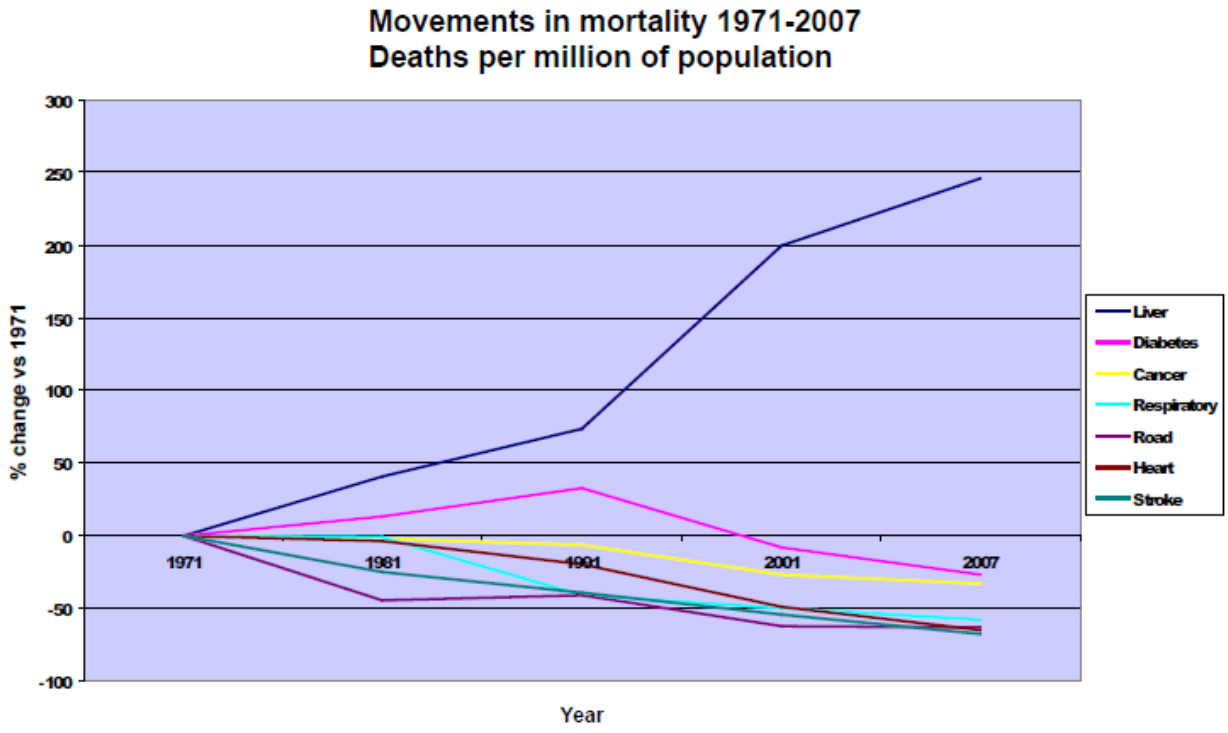
1. BACKGROUND AND RATIONALE

Liver disease is a rapidly rising cause of morbidity and mortality in the western world, being the fifth highest cause of death in the UK and the only one of the top five that continues to rise (Source: Office for National Statistics).

The pathophysiological process of cirrhosis is common to all causes of chronic liver disease and results in (i) a disordered scarred hepatic architecture with associated intrahepatic resistance (portal hypertension) leading to the clinical manifestations of varices, ascites and encephalopathy and (ii) loss of hepatocyte mass resulting in failure of hepatic synthetic function.

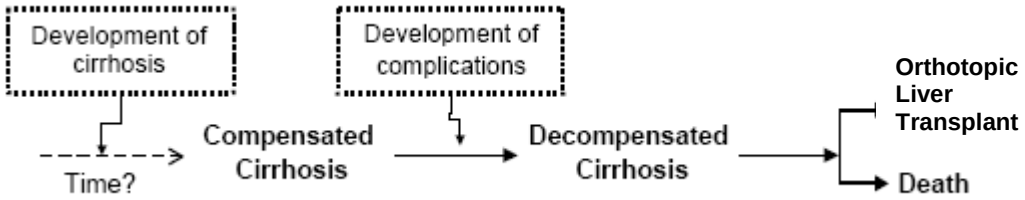
Liver transplantation is currently the only curative treatment available for end stage liver disease. There is a rising demand for transplantation, which has not been matched by an increased supply of donor organs, resulting in significant mortality and morbidity whilst on the waiting list. Transplantation requires lifelong immunosuppression with associated side effects including renal failure, cardiovascular complications and increased risk of malignancy.



Source: British Liver Trust / Office for National Statistics

1.1 Justification for Patient Population

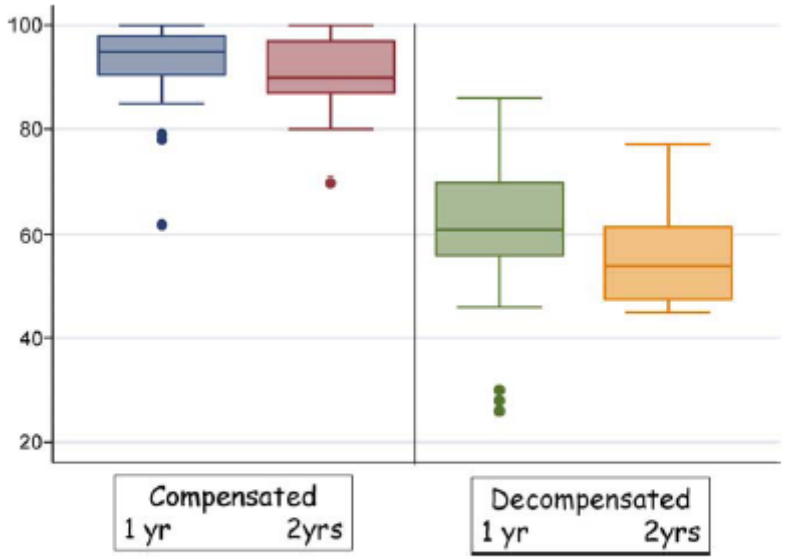
Patients develop cirrhosis at varying rates depending upon the underlying aetiology of the liver disease. Patients with compensated chronic liver disease have advanced fibrosis or cirrhosis but have not yet developed the clinical complications of ascites, varices or encephalopathy.



From D'Amico et al J Hepatol 2006;44:217-231

These complications occur at the rate of 5-7% per year and have devastating implications for prognosis (1). Patients with compensated cirrhosis have a 1-3.4% risk of death at one year (median survival 12 years) and in those with decompensated cirrhosis this rises dramatically to 20-57% risk of death at one year (median survival 2 years).

Furthermore, patients with cirrhosis develop Hepatocellular Carcinoma at a constant rate of around 3% per year (1).



Box plots of one and two year survival rate in patients with compensated and decompensated liver disease

From: D'Amico et al J Hepatol 2006;44:217-231

1.2 Justification for Design

To date there have been 11 published studies investigating the effects of stem cell therapy in liver disease in humans (2). All have been small, non randomised studies and have made no attempt to formally evaluate the effect on either liver disease severity or stage of fibrosis. No studies have sought to identify the mechanism by which adult stem cells exert their effect.

The previous studies have shown that mobilisation of stem cells using Granulocyte Colony Stimulating Factor (G-CSF), harvesting and then re-infusing the cells either peripherally or via the portal vein/hepatic artery, is feasible and safe in patients with chronic liver disease (2).

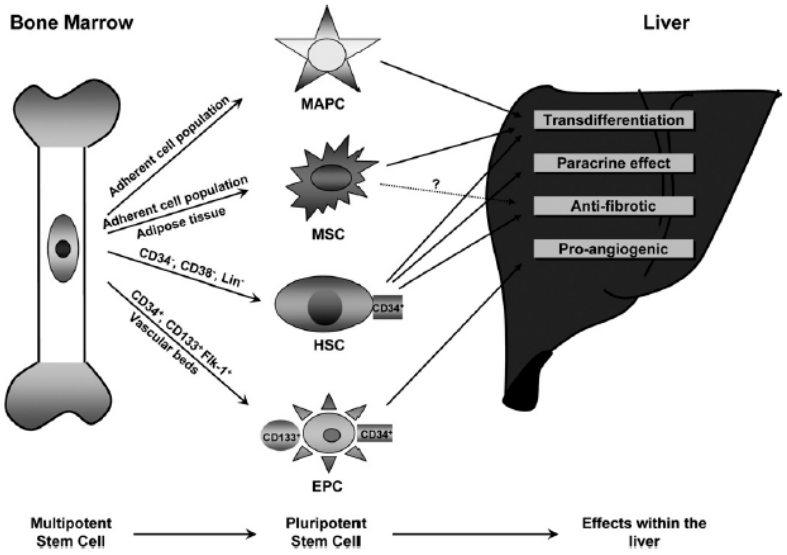
Country	Number of patients	Baseline measures	Source of stem cell	Type of stem cell infused	Number of infused cells	Delivery of stem cells	Improved with infusion?	Follow-up period, mo
Italy[39]	8	CP/MELD	G-CSF mobilization only	N/A	None; 19.33 $CD34^+$ cells/μL in blood 3 days after G-CSF	N/A	Yes	8
Japan[40]	9	CP	Bone marrow from iliac crest (400 mL)	Unsorted MNCs from bone marrow; infused cells were 0.93% $CD34^+/CD45^+/ckit^+$	5.2×10^9 (with 5.2×10^6 $CD34^+/CD45^+/ckit^+$ cells)	Peripheral vein infusion	Yes	6
Iran[41]	4	MELD	Bone marrow from iliac crest (200 mL)	Sorted $CD34^+$ cells	5.25×10^6 isolated and re-infused	Hepatic artery infusion	No	6
Iran[42]	4	MELD	Bone marrow from iliac crest (80–100 mL)	MSCs	31.7×10^6 cells processed and re-infused	Peripheral vein infusion	Yes	12
Brazil[43]	10	Albumin/bilirubin	Bone marrow from iliac crest (50 mL)	Unsorted MNCs	100×10^6 infused	Hepatic artery infusion	No	4
Brazil[44]	30	CP/MELD	Bone marrow from iliac crest (50 mL)	Unsorted MNCs	100×10^6 infused	Hepatic artery infusion	Yes	3
Greece[46]	2	CP/MELD	G-CSF mobilized peripheral blood	Unsorted MNCs containing $CD34^+$ cells	$4–6.93 \times 10^6$ $CD34^+$/kg isolated and infused	Peripheral vein infusion	Yes	12
England[45,67]	5	Albumin/bilirubin	G-CSF mobilized peripheral blood	Sorted $CD34^+$ cells after adherence to plastic	$1 \times 10^6 – 2 \times 10^8$ cells	Hepatic artery (n = 2) or portal vein (n = 3) infusion	No	2
Germany[47]	6	Daily growth rates of hepatic segments	Bone marrow from iliac crest (60–220 mL)	Sorted $CD133^+$ cells	$2.4–12.3 \times 10^6$ infused	Portal vein infusion	Yes	N/A
Germany[48]	13	Daily growth rates of hepatic segments	Bone marrow from iliac crest (60–440 mL)	Sorted $CD133^+$ cells	$2.4–12.3 \times 10^6$ infused	Portal vein infusion	Yes	N/A

From: Houlihan and Newsome Gastroenterology 2008:135 ;438-450

This study will be a phase II randomised controlled trial to examine in greater detail the effect of G-CSF and stem cell therapy on severity of liver disease and attempt to identify the mechanisms by which stem cells produce this effect.

1.3 Choice of treatment

There are two major types of adult bone marrow stem cell – haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), as shown in the figure below.



From: Houlihan and Newsome Gastroenterology 2008:135;438-450

HSCs and Endothelial progenitor cells (EPCs) have been well characterised with respect to their recapitulation of the major haematopoietic lineages and the cell surface markers that can be used to identify and isolate them. Expression of CD34 on the cell surface is used in clinical practice as a marker for a population of cells enriched for stem cells. CD133 represents a more enriched subpopulation of the CD34 cells, with true pluripotent stem cells constituting about 0.1% of a CD133 population.

MSCs and Multi-potent adult progenitor cells (MAPCs) are more difficult to define and constitute a more heterogeneous population. Cell surface expression is not used to identify them, and their identity is confirmed by their adherence to plastic and the ability to differentiate them down chondrocytic and adipocytic cell lineages.(16) MAPCs may contribute to liver regeneration after transdifferentiation to endothelial cells and subsequent neovascularisation of injured tissue(2). MSCs have not been consistently shown to modulate fibrosis, and although they may potentially modulate the fibrotic process by minimising collagen deposition, other data suggest they may contribute to liver fibrosis (17).

Rodent bone marrow cells have been demonstrated to make a significant contribution in animals with chronic liver injury including the stimulation of proliferation of existing hepatocytes, breakdown of scar tissue and to a lesser extent the direct production of new hepatocytes within the liver. Attention initially focussed on the direct stem cell contribution to hepatocyte mass, as demonstrated in the murine model of hereditary tyrosinaemia where an infusion of bone marrow rescued the mice from death due to liver failure. The mechanism by which BM derived hepatocytes are produced is controversial and seems to depend on the mouse model used. In the model of hereditary tyrosinaemia, fusion of BM cells with recipient hepatocytes is the dominant mechanism, whereas in carbon tetrachloride injury and xenotransplantation models transdifferentiation of BM cells into hepatocytes is reported. (18-20).

Subsequently the effect of BM cell infusions on liver scarring has been studied. Rodent BM infusion during chronic liver injury reduced the amount of fibrosis seen, with increased numbers of BM derived MMP-9 expressing cells seen juxtaposed to areas of liver fibrosis.(21)

It has therefore been suggested that HSCs may aid hepatic regeneration through a variety of mechanisms including matrix remodelling, paracrine stimulation of endogenous hepatocytes and the production of new hepatocytes by cellular fusion and transdifferentiation. EPCs may revascularise the injured liver. Expression of growth factors including Hepatic Growth Factor (HGF), Transforming Growth Factor – a (TGF-a), Endothelial Growth Factor (EGF), and Vascular Endothelial Growth Factor (VEGF) promote liver regeneration and hepatocyte proliferation.

On this basis the aim of stem cell therapy in patients with cirrhosis is to **stimulate the regeneration of viable hepatocytes** and **promote the resolution of fibrosis**.

Sources of Stem Cells

BMSC mobilisation into the peripheral circulation through G-CSF administration is an easier and less traumatic procedure for obtaining autologous stem cells, than direct bone marrow harvest by multiple iliac crest aspirations requiring a general anaesthetic. This is particularly so in cirrhotic patients with impaired coagulation and poor clinical condition.

BMSC mobilisation and collection is well established and has become the dominant procedure for obtaining autologous and allogeneic haematopoietic cell grafts.

GCSF

GCSF is currently routinely used in the mobilisation of bone marrow stem cells in the peripheral circulation prior to autologous or allogeneic transplantation.

Animal models of liver injury suggest that BMSC mobilised by G-CSF can lead to liver regeneration and an improvement in liver function (22).

G-CSF is routinely used to mobilise BMSC in the peripheral blood and safety is well established in healthy donors, patients with neoplasms prior to autologous stem cell transplantation and also in patients with heart failure (23 IB9).

A recent dose finding study has shown that G-CSF administered at a dose of 15mcg/kg/day for five days is safe in patients with cirrhosis and is the optimum dose for effective mobilisation without adverse effects (24). This is higher than the routinely used dose of 10mcg/kg/day as patients with cirrhosis demonstrated a poorer mobilisation capacity, possibly related to splenic sequestration and poor BM function. This dosage was tolerated well by patients and no adverse effects were recorded. One previous study using the standard dosage showed a reversible increase in spleen size (25).

CD133+ Bone Marrow Stem Cell mobilisation

CD133 represents a more enriched subpopulation of the CD34 cells, with true pluripotent stem cells constituting about 0.1% of a CD133 population. (26,27).

The presence of CD133 expressing cells in different cell compartments is described below: (28)

	%CD133+	%CD34+	% of CD34+ cells which are CD133+
Bone Marrow	0.52+/-0.11	1.47+/-0.23	36.3+/-2.2
Mobilised Peripheral Blood	1.37+/-0.27	1.75+/-0.31	75.3+/-0.8

G-CSF mobilisation of BMSC into peripheral circulation followed by Leukapheresis has been well tolerated and without adverse effects in small studies of cirrhotic patients. No hepatic complications were seen and no adverse effects on blood coagulation noted. (24)

Data from patients with haematological disorders have demonstrated yields of 2.6-12.2 x 10*6/kg CD133+ cells following a single episode of Leukapheresis and studies in patients with cirrhosis a yield of 1.2x10*6/kg (24).

BMSC Reinfusion

BMSC therapy has been examined in small scale trials in patients with cirrhosis and results have suggested a possible beneficial effect on liver regeneration and improvement in liver function.

A study involving patients with hepatic tumours showed significantly improved hepatic regeneration following the intrahepatic administration of CD133+ BMSC (29).

Improvements in markers of liver function (Serum Bilirubin, Albumin) has also been shown in patients with cirrhosis following infusion of BMSC via a peripheral vein (30,34) or via hepatic artery/portal vein administration (31,32).

Reinfusion of BMSCs has been reported in a variety of different approaches – peripheral vein, direct portal vein application (+/- selective portal vein embolisation) and hepatic artery. No previous studies have examined the efficacy of different methods of delivery. Direct application to either the portal vein or hepatic artery requires expertise and involves potential risk in cirrhotic patients.

Two studies have used peripheral vein delivery and both showed an improvement in liver function following infusion (30,34) however only small numbers of patients were involved. No adverse effects of peripheral vein delivery were observed.

No studies have examined the effect of repeated infusions following a single episode of leukapheresis.

Autologous CD133+ Stem Cells in Cardiac Disease

CD133+ stem cells may contribute to myocardial recovery following acute myocardial infarction, (35) show potent vaso-regenerative capacity (36) and are capable of homing to myocardium in patients with chronic post infarction heart failure.(37)

Trans-epicardial delivery of CD133+ stem cells in conjunction with standard coronary artery bypass grafting (CABG) is safe and feasible (38,39) and trends towards improved heart related functions have been seen.

Patients treated with a combination of trans-epicardial CD133+ stem cell injection, myocardial laser revascularisation and CABG showed a significant improvement in ejection fraction at 12 months follow up (40-42).

A sustained improvement in ejection fraction was seen in patients with ischaemic cardiomyopathy treated with CD133+ stem cells alone, indicating a sole influence of stem cells towards improved cardiac function (41,42).

Large multicentre, phase II/III, randomised controlled trials are currently underway including the TransACT (43) and INSTEM (44) trials using CD133+ stem cells in conjunction with CABG and the REGENERATE-IHD (45) trial using GCSF and CD133+ stem cells in ischaemic heart failure.

2. AIMS, OBJECTIVES AND OUTCOME MEASURES

2.1 Aims and Objectives

This study will evaluate the efficacy and safety of bone marrow stem cell therapy in patients with compensated cirrhosis.

The study will evaluate two different therapies against standard conservative management – (i) administration of G-CSF alone and (ii) administration of G-CSF, isolation of CD133+ BMSC followed by repeated infusions of CD133+ sorted cells.

The Aims of the study are to demonstrate an improvement in liver function and reduction in liver fibrosis in patients receiving these therapies compared with those receiving standard conservative management.

Primary Objective

The primary objective is to demonstrate an improvement in the severity of liver disease over 3 months using either G-CSF alone or G-CSF followed by repeated infusions of HSCs compared with standard conservative management

Secondary Objectives

The secondary objectives are to investigate the effect of either G-CSF alone or G-CSF followed by repeated infusions of CD133+ cells over standard conservative management on :

a) Treatment related adverse events
b) liver fibrosis
c) disease related quality of life
d) liver related clinical events
e) transplant free survival

2.2 Primary Outcome Measure

Primary: Change in MELD score at 90 days from randomisation (delta MELD)

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 patients with cirrhosis undergoing Trans-jugular Intrahepatic Porto-systemic Shunt insertion (3).

MELD has been validated in outpatients with compensated cirrhosis (4) and across a broad spectrum of liver disease (5). It is highly accurate in predicting one week, three month and one year mortality. MELD independently predicts clinical decompensation in patients with compensated cirrhosis (6).

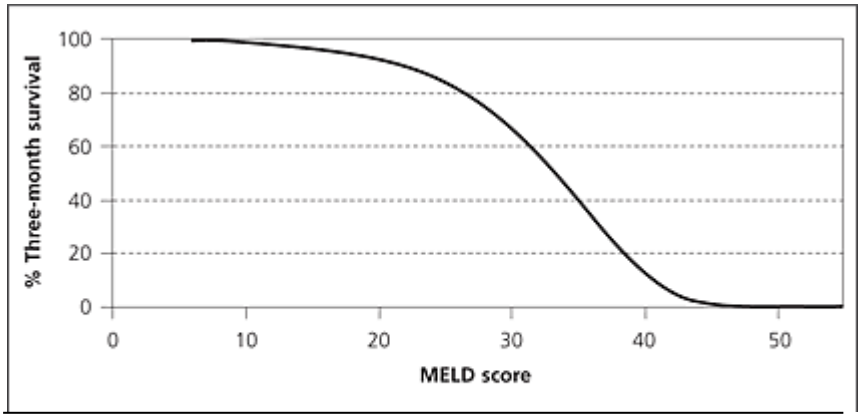
Further studies (7-9) have shown that a change in MELD is a more significant determinant of death than initial MELD alone. Increasing MELD is also associated with the onset of ascites and encephalopathy (9).

For any given MELD, the magnitude and direction of change in MELD score during the previous 30 days was a significant independent mortality predictor (8).

Change in MELD (delta MELD) over 3 months has been shown to be useful in predicting the occurrence of variceal bleeding and encephalopathy.(10)

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 randomisation (Day 1) and Day 90 (Visit 5) 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(mmol/l)*0.011312217)) + (0.378*ln(Bil(mmol/l)*0.058479532)) + (1.12*ln(INR))] + 6.43 (11)

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 patient 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

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 180 and Day 360 will also be tested for Bilirubin, INR and Creatinine to calculate MELD at these time points.

2.3 Secondary Outcome Measures

Transient Elastography (Fibroscan ™, Echosens, France)

A non invasive method for assessing liver fibrosis. Mild amplitude and low frequency vibrations (50 Hz) 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 patients, and has high intra- and inter-observer agreement (12).

It has been validated in most causes of chronic liver disease (13).

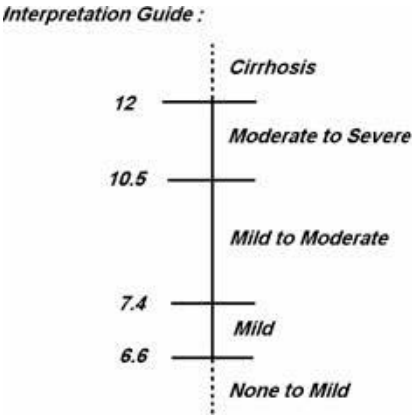
Patients will have a Fibroscan examination performed at randomisation (Day 1), and repeated at Day 90, Day 180 and Day 360.

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)
- Pro-collagen 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 (11) and is a sensitive, specific and reproducible method for the non invasive assessment of hepatic fibrosis (12).



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

ELF is CE marked and fully approved for research use.

Serum samples will be sent for analysis to

iQurLtd
MP 811, Level D South Block
Southampton General Hospital
Tremona Road
Southampton
SO16 6YD

Chronic Liver Disease Questionnaire

This is a liver specific questionnaire for measuring health related quality of life in patients with chronic liver disease (13). 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 patient's global rating scores. All phases of the validation process included patients 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.

Patients will complete a CLDQ at randomisation (Day 1) and Day 90, Day 180 and Day 360.

United Kingdom End stage Liver Disease Score (UKELD)

A scoring system developed by the UK Liver Transplant Units to predict transplant waiting list mortality (15).

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

UKELD = (5*((1.079*ln_INR)+(0.297*ln_creatinine)+(0.626*ln_bilirubin)-(16.313*ln_sodium))+435)

INR, serum creatinine and bilirubin values less than 1 are capped at 1.

Serum creatinine values greater than 400 are capped at 400.

Sodium values outside the range 112 to 150 are capped at the lower (112) and upper (150) limits

Patients will have UKELD score calculated at randomisation (Day 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 for Bilirubin, Albumin, INR, Platelet count, ALT, AST, ALP, GGT and AFP at randomisation (Day 0) and Day 5, Day 30, Day 60, Day 90, Day 180 and Day 360.

Levels of Circulating CD133+ cells and Circulating CD34+ cells will be measured in patients in Group 2 and 3 at randomisation (Day 1) and on teh 5th Treatment day (end of GCSF treatment) Day 5.

Individual Blood Parameters will be measured in CPA accredited laboratories at trial sites.

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	Requiring introduction of treatment or hospitalisation
Portal Hypertensive Bleeding	Confirmed at endoscopic examination
Spontaneous Bacterial Peritonitis	PMN cell count >250 cm3 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 randomisation and the date of transplant or death from any cause. Surviving transplant free patients will be censored at date last seen alive.

3. ELIGIBILITY

3.1 Inclusion Criteria

1. 18≤ AGE ≤75 at randomisation

2. 11.00 ≤ MELD ≤15.50 at randomisation

3. Aetiology of liver disease, one or more of :

Alcohol related Liver Disease
- Features (clinical, biochemical, histological or radiological) of chronic liver disease with a compatible history of alcohol excess (>80g/day), in the absence of other causes of chronic liver disease
- Abstinent >6 months prior to enrolment

Hepatitis C
- Positive HCV Antibody
- Not currently on antiviral therapy

Hepatitis B
- Positive HBsAg and Anti-HBc
- Established on antiviral therapy with adequate viral suppression

Primary Biliary Cirrhosis
- 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

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

Cryptogenic cirrhosis
- Diagnosis of cirrhosis unattributable to any other cause

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

Alpha-1 Antitrypsin Deficiency
- Diagnosis based on compatible genetic, phenotypic or histological testing.

4. Cirrhosis, defined as one of:

- Previous Liver Biopsy confirming histological features of cirrhosis
- Transient Elastography (Fibroscan) > 18 kPa
- Clinical and Radiological features that in the opinion of the investigator correlate with a diagnosis of cirrhosis
- AST:Platelet Ratio Index (APRI) > 2.0
  (APRI = (([AST]/ULN)*[Plt])x100)

3.2 Exclusion Criteria

- Refusal or inability to give informed consent to participate in the study
- Average alcohol ingestion >21 units/week (male) / >14units/week (female)
- 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.
- Ascites — Unless, in the opinion of the investigator, the ascites is minimal and well controlled with no changes to diuretic therapy in last 3 months
- Encephalopathy — Current or requiring hospitalisation for treatment in last 3 months.
- Portal Hypertensive Bleeding — Active episode of bleeding requiring treatment or Hospitalisation in the 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 Liver Transplant
- Listed for Liver Transplantation
- Recent history of pulmonary infiltrates or pneumonia: patients should have completely recovered from any previous episodes, both clinically and radiologically.
- 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

- Participation in any clinical study of an investigational agent within 30 days of randomisation.

- Presence of clinically relevant cardiovascular, pulmonary, gastro-intestinal, renal, metabolic, haematological, neurological, psychiatric, systemic, ocular, gynaecological or any acute infectious disease or signs of acute illness that in the opinion of the investigator might compromise the patient's safe participation in the study

- Presence or history of cancer within past 5 years with exception of adequately treated localised basal cell carcinoma of the skin, in situ cervical cancer or solid malignancy surgically excised in total without recurrence for 5 years

- Pregnancy or Breastfeeding

    - Women of child-bearing potential and men who have partners of child-bearing potential who are not willing to practise effective contraception for the duration of the study and for twelve months (females) and six months (males) after the last study drug administration.

4. TRIAL DESIGN

This is a multi centre, early phase, open label, randomised controlled trial comparing standard conservative management of patients with i) GCSF alone and ii) GCSF followed by Leukapheresis, CD133+ cell isolation and autologous repeated infusions of CD133+ bone marrow stem cells.

The study will consist of three stages:

Stage 1	Screening and Enrolment	
Stage 2	Treatment	up to Day 90 (Primary Endpoint) from randomisation
Stage 3	Follow up	up to one year (Secondary Endpoints) from randomisation

Each patient will be followed in the trial for one year or until listed for transplantation or death.

At randomisation, patients will be assigned to one of three trial groups:

Group 1	Control group, Standard conservative management
Group 2	Treatment: GCSF Alone
Group 3	Treatment: GCSF followed by Leukapheresis, CD133+ cell isolation and repeated infusions on days 5/6, 30, 60 via peripheral vein

Screening, Treatment and Follow up visits will be scheduled as described in Sections 5,6 and 7.

A summary of the trial is displayed in Figure 1.

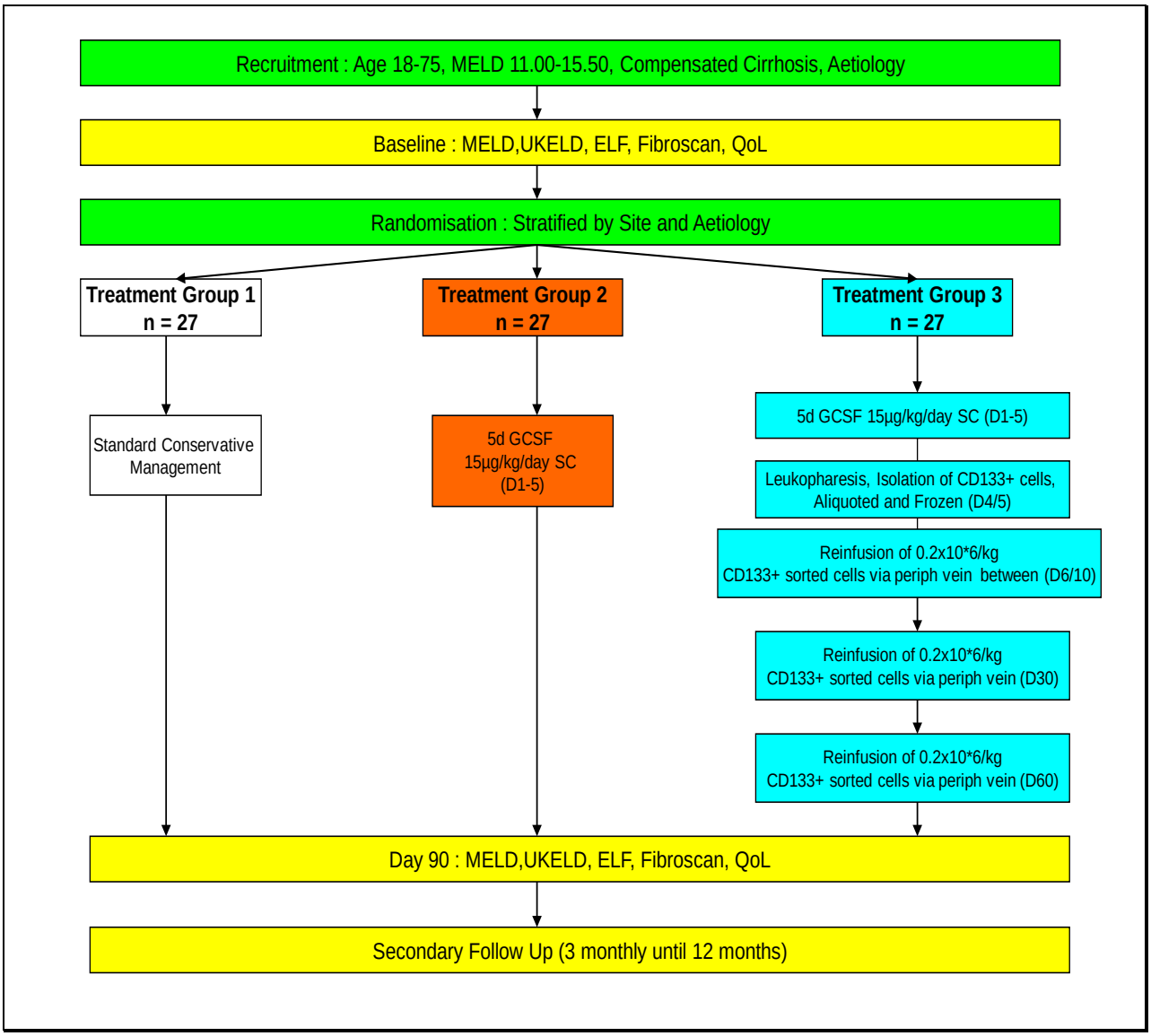


Figure 1: Outline of REALISTIC Trial

5. SCREENING AND CONSENT

5.1 Pre Screening

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.

At an initial meeting the trial will be introduced and explained to the potential participant, written patient information will be provided and there will be an opportunity for the potential participant to ask questions.

At the end of this initial meeting, 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.

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

5.2 Informed Consent

The Investigator (or designated co investigator as documented on the Signature and Delegation log) will obtain written informed consent for each patient prior to performing any trial related procedure. A Patient Information Sheet will be provided to facilitate this process. The Investigator will ensure that they adequately explain the aim, trial treatment, anticipated benefits and potential hazards of taking part in the trial to the patient. The Investigator should also stress that the patient is completely free to refuse to take part or withdraw from the trial at any time.

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

If the patient expresses an interest in participating in the trial 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 given to the patient, a copy should be filed in the hospital notes, and the original placed in the Investigator Site File (ISF). Once the patient is entered into the trial the patient's trial number will be entered on the Informed Consent Form maintained in the ISF. In addition, after the patient has been entered into the clinical trial and with the patient's prior consent, a copy of consent form will be sent to the central coordinating centre.

Details of the informed consent discussions should be recorded in the patient's medical notes, this should include date of, and information regarding, the initial discussion, the date consent was given, with the name of the trial and the version number of the Patient Information Sheet and Informed Consent Form. Throughout the trial the patient should have the opportunity to ask questions about the trial and any new information that may be relevant to the patient's continued participation should be shared with them in a timely manner. On occasion it may be necessary to re-consent the patient in which case the process above should be followed and the patient's right to withdraw from the trial respected.

Details of all patients approached about the trial should be recorded on the Patient Screening/Enrolment Log and with the patient's prior consent their General Practitioner (GP) should also be informed that they are taking part in the trial. A GP Letter is provided electronically for this purpose.

5.3 Screening

Screening Visit (Visit 1):

The screening visit should occur within 14 days prior to study enrolment. All tests and procedures should be performed within seven days prior to study randomisation. If required some screening procedures can be performed on the actual day of randomisation.

No trial specific tests or interventions which don't form part of routine standard practice should be conducted prior to the patient providing written informed consent.

- Review with patient 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 patient (see section 5.2)) the following screening investigations will take place.

- Patient demographics recorded and patient registered

- 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)

- Full clinical examination
- Record HR, BP
- Measure Height, Weight – calculate BMI

- Obtain blood
  - 1. Baseline investigations
    - FBC
    - INR
    - U&E
    - LFT
    - AFP
    - Serum for storage
    - Serum for ELF panel
  - 2. Liver Screen (if no previous result available)
    - HbsAg
    - HCV Ab
    - AMA / SMA / Ig's
    - Ferritin
    - Caeruloplasmin
    - A1AT
- Obtain serum for ELF panel and storage

- Obtain 12 lead ECG

- Obtain Baseline Abdominal USS and Transient Elastography
- Perform Baseline CLDQ questionnaire
- Perform Urinary Pregnancy Test in Women of child bearing potential

5.4 Mandatory Microbiology

As part of the screening procedure all patients will require mandatory testing for blood borne infectious agents as per National Blood Service requirements for screening of blood products prior to processing and storage.

These tests must be performed within 30 days of Leukapheresis and consist of serological testing for HBV, HCV, Human Immunodeficiency Virus (HIV), Human T-Lymphotropic Virus 1 and 2 (HTLV-1, HTLV-2) and Syphilis.

Appropriate pre-test counselling 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.

6. TRIAL ENTRY

6.1 Confirmation of Eligibility

Once the results of the screening visit are available (usually the same day) the following will be checked:

- Patient Consent completed
- Confirm all Inclusion Criteria: 18 ≤ AGE ≤ 75

  11.00 ≤ MELD ≤15.50

  Diagnosis of Cirrhosis

  Aetiology of Liver Disease
- Review of Exclusion Criteria

6.2 Randomisation

Patients will be randomised to one of three treatment groups:

Group 1 Control group, No specific treatment, and Standard supportive management

Group 2 Treatment: GCSF Alone

Group 3 Treatment: GCSF followed by Leukapheresis, CD133+ cell isolation and

Repeated infusions: 1) one infusion between days 5 -10. 2) one infusion on Day 30 and 3) One infusion on Day 60 via peripheral vein

Patients will be randomly assigned to treatment on a 1:1:1 based on a minimisation algorithm prepared by the CRCTU Programming team and tested by the trial statistician. Randomisation will be stratified by (i) randomising centre and (ii) aetiology of disease (ALD, HCV, Other). When a patient falls into 2 or more strata then the dominant aetiology (as determined by treatment physician) will be used.

At randomisation, patients will be allocated a unique patient trial number and scheduled for treatment and follow up visits as detailed in section 7.

Contact Details for Randomisation:

CRUK Clinical Trials Unit
School of Cancer Sciences
The University of Birmingham
Edgbaston
Birmingham
B15 2TT

Phone: 0800 371 969
(Mon – Fri, 9am – 5pm)
General enquires: 0121 414 8255
Fax: 0121 414 3700

7. TREATMENT DETAILS

7.1 Medication preparation

- Trade name : ***Granocyte*** (Chugai Pharma UK Ltd)
  Active Substance : **Lenograstim** (Recombinant Human Granulocyte-Colony Stimulating Factor (rHuG-CSF))

Granocyte® (Lenograstim) will be supplied by Chugai Pharma UK. This will be packaged and labelled in the standard manner according to current Marketing Authorisation. Trial specific labelling will be added by the hospital pharmacy at the trial site to ensure that the requirements of Annex 13 (Manufacture of investigational medicinal products) of EU Good Manufacturing Practice guidance (July 2003). The medication will be stored and dispensed by the hospital pharmacy at the trial site.

Supplied as either Granocyte 13.4 million International Units (equivalent to Lenograstim 105 µg) per ml after reconstitution or Granocyte 33.6 million International Units (equivalent to Lenograstim 263 µg) per ml after reconstitution.

Packaged in the form of powder and solvent for injection (ampoule or pre-filled 1ml syringe).

Administered subcutaneously, by trial staff or other qualified personnel only..

Dosage to be used in this study will be 15µg/kg/day. This is higher than the standard dose and has been shown to be safe and more effective in patients with cirrhosis(24).

A full Summary of Product Characteristics and Patient Information Leaflet are available and supplied to Investigators.

- Isolated CD133+ Bone Marrow Stem Cells

Leukapheresis is performed according to trial site standard protocol.

Cell isolation is performed using CliniMACS technology (Miltenyi Biotec GmBH, Germany).
The CliniMACS Plus instrument is able to perform clinical scale magnetic enrichment of target cells or depletion of unwanted cells in a closed and sterile system.
The harvested leukocytes are magnetically labelled using a CD133+ specific reagent consisting of super paramagnetic iron dextran particles directly conjugated to CD133 antibodies.
The cells are then applied to the selection column of the CliniMACS instrument followed by washing and the cells are eluted from the column.

The CliniMACS system provides CD133+ cells with high purity and yield, and passive depletion of unwanted cells.
Enrichment results from Peripheral Blood:

	n=11	n=84	n=12	(46-48)
Purity CD133 (%)	94	93	97	
Recovery CD133 (%)	69	81	69	
CD3 log depletion	4.2	3.8	N/A	

The CliniMACS Plus instrument is CE-marked for clinical use in Europe.
The CliniMACS system components are manufactured and controlled under an ISO 13485 certified quality system.

The dosage of CD133+ cells is 0.2 x 10*6 cells/kg for each of three infusions at monthly intervals. This regime would require the collection of a minimum of 0.6x10*6 cells/kg.
A recent study examining the effectiveness of peripheral blood stem cell mobilisation and harvesting in cirrhotic patients showed a mean number of 1.2 +/- 0.5 x 10*6 cells/kg were collected (24).

Isolated CD133+ cells are aliquoted in three portions in the required quantities, one portion will be available for immediate reinfusion and a further two portions will be cryopreserved according to standard protocols for later reinfusion.

CD133+ cell reinfusion is performed according to standard protocol for the reinfusion of Autologous Stem Cells.

The Procurement, Processing, Storage and Distribution of the Autologous CD133+ Stem Cells will be performed and licensed in accordance with the Quality and Safety Regulations of the Human Tissue Authority.

7.2 Dose Modifications and Toxicity management recommendations

There will be no dose reductions permitted during the course of study therapy (Groups 2 +3) in relation to Granocyte® (Lenograstim) administration. If possible, toxicities should be managed symptomatically. If toxicity occurs the appropriate treatment will be used to ameliorate signs and symptoms. In the event of uncontrollable toxicity or development of some some specific toxicities (detailed below), the patient may be withdrawn from study therapy immediately. The recommended management and appropriate actions in relation to some specific toxicities / adverse reactions is detailed below;

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.

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.

Bone Pain

A stepwise approach to analgesia for Lenograstim related bone pain is recommended.

- Simple non opioid analgesia (eg Paracetamol 1g qds/prn)
- Mild opioid analgesia (eg addition of Codeine Phosphate 30-60mg qds or change to Co-codamol equivalent)
- Stronger Analgesia (eg Tramadol 50-100mg qds) reserved for severe cases

Dosage of analgesics may need adjusting depending on hepatic and renal function.

Non Steroidal Anti Inflammatory Analgesics (NSAIDS) are not recommended.

Abdominal Pain

The onset of abdominal pain or referred abdominal pain (eg shoulder tip pain) during the treatment phase of the study should raise the suspicion of actual or potential Splenic Rupture.

An Abdominal Ultrasound scan is routinely performed on patients in Groups 2 and 3 on Day 4, however development of symptoms should prompt urgent investigation.

A comprehensive clinical assessment followed by an **Urgent Abdominal Ultrasound scan** and if necessary Abdominal CT scan to assess for potential Splenic Rupture should be performed as soon as possible.

Investigations appropriate to the symptoms should also be arranged, including but not limited to blood tests, urinalysis, plain X-Rays, further imaging – allowing diagnosis and management of other conditions.

Further action is dependent on the results of these investigations:

- Suspected or Confirmed Splenic Rupture – Immediate withdrawal of treatment, Hospitalisation and Specialist Surgical Input for Further Management
- Alternative Abdominal Pathology – Specialist Input and Management appropriate to diagnosis, Withdrawal of treatment at discretion of Investigator
- No Evidence of Splenic Rupture – Manage symptomatically, Withdrawal of treatment at discretion of Investigator

Leucocytosis

Lenograstim should be discontinued if the peripheral blood White Cell Count is greater than $70x10^9$/l at any stage during treatment.

No specific treatment is usually required unless:

- Associated with specific symptoms or signs
- Persistent and unrelated to study treatment.

Specialist Haematological advice should be sought as needed.

Thrombocytopenia

The development of thrombocytopenia is usually a delayed reaction seen after completion of Lenograstim therapy or following Leukapheresis.

No specific treatment is usually required unless:

- Associated with specific symptoms or signs of bleeding
- Persistent and unrelated to study treatment.

Specialist Haematological advice should be sought as needed.

Pulmonary Symptoms

The onset of pulmonary symptoms or signs, such as cough, fever and dyspnoea, in association with radiological signs of pulmonary infiltrates and deterioration in pulmonary functions may be preliminary signs of Acute Respiratory Distress Syndrome (ARDS)

A comprehensive clinical assessment followed by **Urgent Chest X-Ray** should be performed as soon as possible, further investigations including but not limited to blood tests, sputum analysis and further imaging may also be required.

Further action will depend on the results of these investigations:

- Evidence of Pulmonary Infection / Infiltrates – Immediate withdrawal of treatment, Specialist Respiratory Input for Further Management
- No evidence of Pulmonary Infection / Infiltrates – Withdrawal of treatment at discretion of Investigator

Skin Reactions / Injection Site Reactions

Skin reactions should be avoided by rotating injection sites and allowing the injection to warm to room temperature prior to injecting.

Cooling of the skin and application of topical antihistamines may be used to alleviate the effects of such reactions.

In the case of severe or persisting symptoms, localised infection should be excluded.

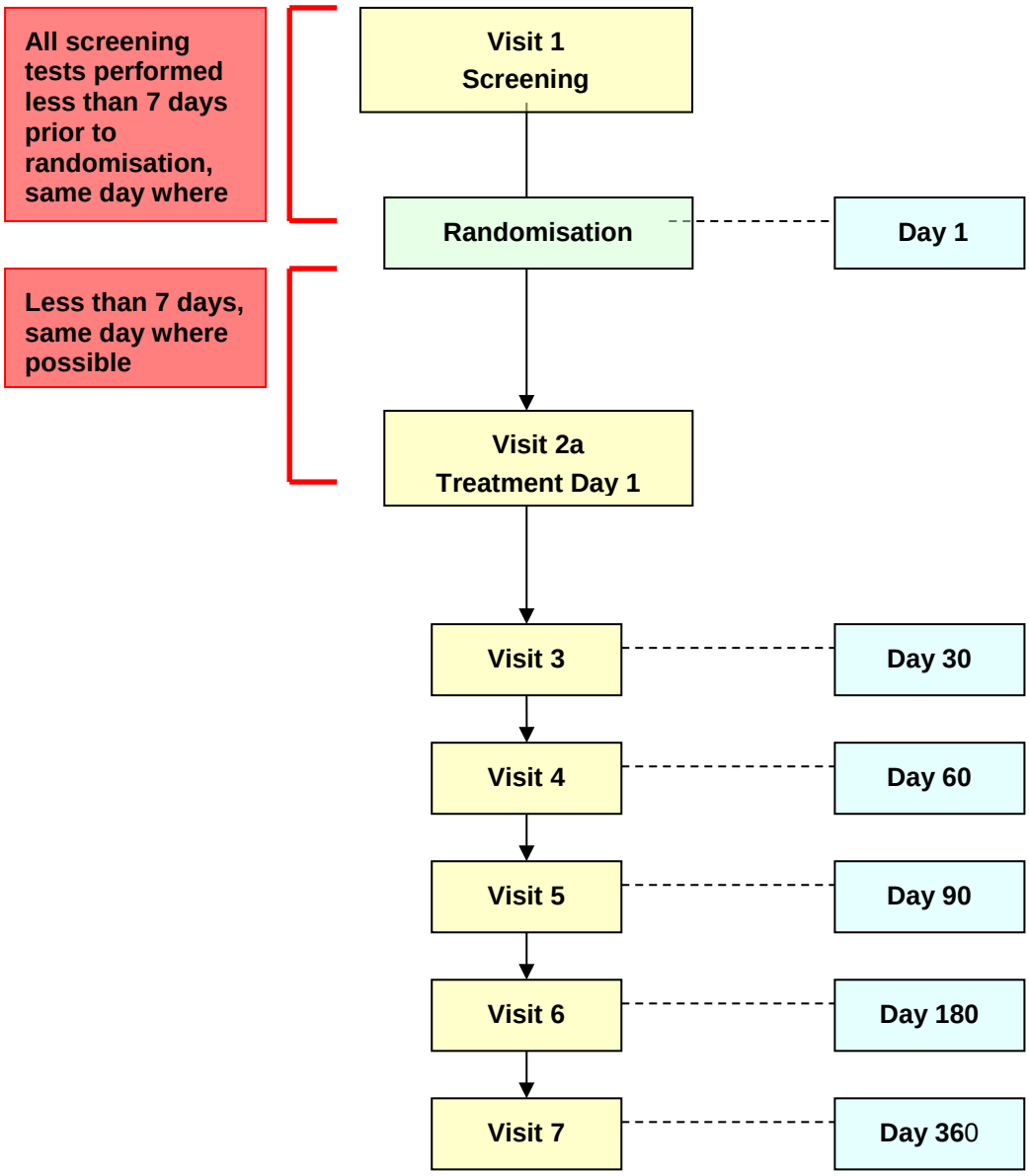
7.3 Treatment Scheduling

Treatment schedule (day 1 – day 90) will be dependent on allocated treatment group.

Initial treatment visit will be scheduled for a mutually convenient date for both patient and investigator. This can be scheduled for the same day as the screening visit and proceed if patient enrolled.

As far as is practically possible the initial treatment visit should take place no longer than seven days following the screening visit and randomisation. If trial site scheduling does not permit this, then treatment should commence as soon as practically possible. Day 30, Day 60 and Day 90 treatments/assessments will remain fixed with respect to Day 0 (randomisation), however a margin of +/- 3 days is permitted to allow convenient scheduling.

Scheduling Timeline:



7.4 Treatment Schedule Trial Group 1 – Control Group

Visit 2 (Day 1)

- 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
- Schedule Visit 3 for 30 days +/- 3 days from actual date randomisation (Day 1)

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
- Obtain serum for ELF panel and storage
- Schedule Visit 4 for 30 days +/- 3 days from actual date of Visit 3

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
- Obtain serum for ELF panel and storage
- Schedule Visit 5 for 60 days +/- 3 days from actual date of Randomisation (Day 1)

7.5 Treatment Schedule Trial Group 2 – G-CSF Alone

Visit 2a (1ST Treatment Day)

- 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
- Obtain blood for FBC, Circulating CD133+ and Circulating CD34+ cell concentrations (prior to GCSF administration)
- Administer 15µg/kg Granocyte (Lenograstim) subcutaneously
- Observe for 10 minutes post injection
- Schedule Visit 3 for 30 days +/- 3 days from actual date of randomisation (Day 1)

Visit 2b (2nd Treatment Day)

- (Inquire regarding adverse events)
- Administer 15µg/kg Granocyte (Lenograstim) subcutaneously
- (Observe for 10 minutes post injection)

Visit 2c (3rd Treatment Day)

- (Inquire regarding adverse events)
- Administer 15µg/kg Granocyte (Lenograstim) subcutaneously
- (Observe for 10 minutes post injection)

Visit 2d (4th Treatment Day)

- (Inquire regarding adverse events)
- Administer 15µg/kg Granocyte (Lenograstim) subcutaneously
- Obtain blood for FBC
- (Observe for 10 minutes post injection)

Visit 2e (5th Treatment Day)

- (Inquire regarding adverse events)
- Administer 15µg/kg Granocyte (Lenograstim) subcutaneously
- Obtain blood for FBC, Circulating CD133+ and Circulating CD34+ cell concentrations
- Observe for 10 minutes post injection
- Perform Abdominal USS to assess spleen size

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
- Obtain serum for ELF panel and storage
- Schedule Visit 4 for 60 days +/- 3 days from actual date of Randomisation

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
- Obtain serum for ELF panel and storage
- Schedule Visit 5 for 90 days +/- 3 days from actual date of Randomisation (Day 1)

7.6 Treatment Schedule Trial Group 3 – G-CSF and Repeated Stem Cell Infusion Group

Visit 2a (1st Treatment Day)

- 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
-
- Obtain blood for FBC, Circulating CD133+ and Circulating CD34+ cell concentrations (prior to GCSF administration)
- Administer 15µg/kg Granocyte (Lenograstim) subcutaneously
- Observe for 10 minutes following injection
- Schedule Visit 3 for 30 days +/- 3 days from actual date of randomisation (Day 1)

Visit 2b (2nd Treatment Day)

- (Inquire regarding adverse events)
- Administer 15µg/kg Granocyte (Lenograstim) subcutaneously
- (Observe for 10 minutes post injection)

Visit 2c (3rd Treatment Day)

- (Inquire regarding adverse events)
- Administer 15µg/kg Granocyte (Lenograstim) subcutaneously
- (Observe for 10 minutes post injection)

Visit 2d (4th Treatment Day)

- (Inquire regarding adverse events)
- Administer 15µg/kg Granocyte (Lenograstim) subcutaneously
- Obtain blood for FBC
- (Observe for 10 minutes post injection)

Visit 2e (5th Treatment Day)

- (Inquire regarding adverse events)
- Administer 15µg/kg Granocyte (Lenograstim) subcutaneously
- Obtain blood for FBC, Circulating CD133+ and Circulating CD34+ cell concentrations
- Perform Abdominal USS to assess spleen size
- Leukapheresis performed according to trial site standard protocol.

Visit 2f (6th Treatment Day)

If insufficient CD133+ cells have been harvested from Leukapheresis on Day 5 then a further Leukapheresis will be performed on Day 6 and cells will be re-infused on Day 6.

- Leukapheresis performed according to trial site standard protocol (only if required, due to insufficient cell collection on Day 5).
- Isolated CD133+ cells (0.2 x 10*6 cells/kg) re-infused via peripheral vein according to trial site standard protocol.(see note below concerning CD133+ cell infusions)

Reinfusion of CD133+ cells

The actual timing of the first reinfusion of CD133+cells will be determined by the actual number of cells collected from each individual patients on day 5 and procedure scheduling / time constraints. The first reinfusion of cells will occur between Day 6 (visit 2f) and day 10. If a repeat Leukapheresis is required on Day 6 then the re-infusion of CD133+ cells will occur between day 6 and day 10. AFTER the leukapheresis procedure on Day 6.

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
- Obtain serum for ELF panel and storage
- Isolated CD133+ cells (0.2 x 10*6 cells/kg) re-infused via peripheral vein according to trial site standard protocol.
- Schedule Visit 4 for 60 days +/- 3 days from actual date of Randomisation ((Day 1)

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
- Obtain serum for ELF panel and storage
- Isolated CD133+ cells (0.2 x 10*6 cells/kg) re-infused via peripheral vein according to trial site standard protocol.
- Schedule Visit 5 for 90 days +/- 3 days from actual date of Randomisation (Day 1)

7.7 Participant Follow Up

The schedule of visits during the follow up phase (Day 90 to Day 360) of the study will be the same for all trial groups.

Visit 5 (Day 90)

- 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, AFP
- Obtain Serum for ELF panel and storage
- Perform Fibroscan™ examination
- Complete repeat CLDQ questionnaire
- Schedule Visit 6 for 180 days +/- 3 days from actual date of Randomisation (Day 1)

Visit 6 (Day 180)

- 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, AFP
- Obtain Serum for ELF panel and storage
- Perform Abdominal USS
- Perform Fibroscan™ examination
- Complete repeat CLDQ questionnaire
- Schedule Visit 7 for 360 days +/- 3 days from actual date of Randomisation (Day 1)

Visit 7 (Day 360) – Final Trial Visit

- 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, AFP
- Obtain serum for ELF panel and storage
- Perform Fibroscan ™ examination
- Complete repeat CLDQ questionnaire

7.8 Unscheduled Visits

On enrolment participants will be provided with contact details (telephone, e-mail) for trial staff who can be contacted for advice.

In cases of suspected clinical events or adverse events, an unscheduled visit can be arranged to assess the participant:

Unscheduled Visit

- 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, AFP
- Consider expert evaluation as needed

7.9 Treatment compliance

Stem Cell infusions require administration by trial staff and therefore will be directly observed.

All administrations of GCSF will be performed by trial staff and participant refusal of medication will be directly monitored by trial staff.

7.10 Treatment Discontinuation

Treatment with Lenograstim should be discontinued immediately in the event of any of:

- The onset of pulmonary symptoms or signs (eg cough, fever, dyspnoea), in association with either radiological signs of pulmonary infiltrates or deterioration in pulmonary function
- Peripheral Blood Leukocyte count > 70 $x10^9$/l

7.11 Concomitant Therapy

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 exceptions:

- The introduction of antiviral therapy for HCV
- Changes to antiviral therapy for HBV
- The introduction of Ursodeoxycholic Acid for patients with PBC
- Participation in another trial of an investigational product

7.12 Participant Withdrawal

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 patient 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 patient attendance failure and persistent non-compliance)
- withdrawal by the Investigator for clinical reasons not related to the study drug treatment

Participant withdrawals will not be replaced. 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

Patients may withdraw consent at any time during a trial. The details of withdrawal should be clearly documented and communicated to the Trial Office.

The following should be clearly documented in the medical notes:

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

8. ADVERSE EVENT REPORTING

8.1 Reporting Requirements

All Adverse Events, whether observed by the investigator or reported by the patient during the study period and whether or not they are considered related to the study treatment, must be recorded in the CRF including events associated with the deterioration of underlying chronic liver disease. The NCI CTC 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' in the CRF (refer to the CTC guide for grading).

A non-leading, open question will be asked initially to evaluate for possible adverse events.

For example:

At screening / baseline - 'Are you experiencing any symptoms?'
At subsequent visits – 'How have you been since your last visit?'

Pre-existing conditions

A pre-existing condition must not be reported as an AE unless the condition worsens by at least one CTC grade during the trial. The condition, however, must be reported in the CRF.

8.2 Reporting Period

The reporting period for AEs will commence from date of consent and will continue until resolution or 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.

The reporting period for serious adverse events (SAE's) is from the date of consent until 30 days after last possible CD133+ cell infusion i.e.; Day 90 visit (Treatment group 3). 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 insure that the actual reporting period for SAE events is the same for each treatment group.. If a patient is withdrawn from study prior to day 90 then all SAE's will be reported until 30 days post last study infusion or CD133+ infusion. For patients within group 1 (Non-therapy group) who withdraw before day 90 no SAE events will be recorded after the date of withdrawal from the study. Any SAE events with a start date that is the same as, or prior to the patient being withdrawn from study will be followed until resolution.

8.3 Definitions

European Directive 2001/20/EC

- Adverse Event

Any untoward medical occurrence in a participant administered a medicinal product and which does not necessarily have a causal relationship with this treatment.

An adverse event can be any unfavourable and unintended sign, symptom or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product.

- Adverse Reaction

All untoward and unintended responses to an investigational medicinal product related to any dose administered. All adverse events judged by either the reporting investigator as having a reasonable causal relationship to a medicinal product qualify as adverse reactions.

- Unexpected Adverse Reaction

An adverse reaction, the nature, or severity of which is not consistent with the applicable product information (eg SmPC). When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.

Serious Adverse Event or Serious Adverse Reaction

Any untoward medical occurrence or effect that:

Results in death

Is life threatening*

Requires hospitalisation or prolongation of an existing hospitalisation**

Results in persistent or significant disability or incapacity

Is a congenital anomaly or birth defect

Or is otherwise considered medically significant by the Investigator***

*Life threatening in the definition of serious adverse event refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event which might have caused death if it was more severe.

** Hospitalisation is defined as an unplanned, overnight, formal inpatient admission, even if the hospitalisation is a precautionary measure for continued observation. Thus hospitalisation for protocol treatment, elective procedures (unless brought forward due to worsening symptoms) or for social reasons are not regarded as a SAE.

*** Medical judgement should be exercised in deciding whether an AE is serious in other situations. AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the SAE definition above, should be considered serious.

If a patient dies as a result of a SAE, any post-mortem findings including histopathology must be provided.

8.4 Assessment of adverse events

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). For events not listed in the toxicity table, severity should be recorded as;

Mild Subject is aware of the event or symptom, but the event or symptom is easily tolerated.

Moderate Subject experiences sufficient discomfort to interfere with or reduce their usual level of activity.

Severe Significant impairment of functioning, subject is unable to carry out usual activities.

Life threatening Risk of death, organ damage or disability

Relationship to study therapy will be assessed using the following definitions.

Definitely:

- starts a reasonable time after the study drug administration,
- stops/ improves when the study drug has been stopped,
- can reasonably be explained by known characteristics of the study drug

Probably:

- starts a reasonable time after the study drug administration,
- stops/ improves when the study drug has been stopped,
- cannot be reasonably explained by known characteristics of the patient's clinical state

Possibly:

- starts a reasonable time after the study drug administration but
- could have been produced by the subject's clinical state or other modes of therapy administered to the patient

Unlikely to be related:

- The time association or the patient's clinical state is such that the study drug is not likely to have had an association with the observed effect.

Unrelated:

- The AE is definitely not associated with the study drug administered.

8.5 Reporting of adverse events and serious adverse events

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 patient's CRF.

Serious adverse events

All SAEs must be reported to the Cancer Research UK Clinical Trials Unit (CRCTU) **within 24 hours** of the Investigator becoming aware of the event. SAEs must be documented on the SAE Form, which must be faxed within 24 hours to CRCTU on the number below.

SAE FAX NO: 0121 414 8286

The form should be completed and signed by the responsible investigator and faxed to the trial office immediately. In signing off the SAE form the investigator is confirming the causality assessment.

All serious AEs still present at the end of the study must be followed at least until the final outcome is determined, even if it implies that the follow-up continues after the patients leave the trial and when appropriate until the end of the planned period of follow-up.

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On receipt of a SAE form seriousness and causality will be determined by a Clinical Coordinator. A SAE judged by the Investigator or Clinical Coordinator to have a reasonable causal relationship with the trial medication will be regarded as a SAR. The Clinical Coordinator will also assess all SARs for expectedness. If the event meets the definition of a SAR that is unexpected in nature it will be classified as a SUSAR.

All SAE forms received by the study office will receive acknowledgment of the SAE Report via a receipt fax. This communication will also will detail any missing information. At this time a unique reference number will be assigned to the SAE and should be used in all future correspondence with the trials office.

8.6 Reporting of events to other organizations

Regulatory Authorities and Main research Ethics Committee

SUSARs

A SUSAR is a Suspected, Unexpected Serious Reaction. A SUSAR fulfils the definition of a SAE, is suspected to at least be possibly related to the investigational product and is considered to be unexpected according to the relevant product information. SUSARs will be identified by the Chief Investigator or delegate promptly from SAEs reported. SUSARs will be identified in sufficient time to allow reporting to the MHRA and Main REC within the timeframes specified below.

It is the responsibility of the CRCTU to report all SUSARs to the MHRA and Main REC. Fatal or life-threatening SUSARs must be reported to the MHRA and Main REC within 7 calendar days of CR CTU becoming aware of the event. Additional information regarding fatal or life-threatening SUSARs must be reported to the MHRA and Main REC within 8 calendars days of submitting the report. All other SUSARs are reportable within 15 calendar days of CRCTU becoming aware of the event with additional information being reported as soon as it becomes available.

SARs

The Trials Office will report details of all SARs (including SUSARs) to the MHRA and Main REC annually, from the date of the Clinical Trial Authorisation, in the form of an Annual Safety Report.

AEs

Details of all AEs experienced during the reporting period of the clinical trial will be reported to the MHRA on request.

Other Safety Issues Identified During the Course of the Trial

The MHRA and main REC will be notified immediately if a significant safety issue is identified during the course of the trial.

Investigators

Details of all SUSARs and any other safety issue which arises during the course of the trial will be reported to all Investigators.

Independent Data Monitoring Committee

An Independent Data Monitoring Committee will review all SAEs annually.

Chugai Pharma UK Limited

All SAEs classified as “possibly related”, “probably related” or “definitely related” to Granocyte ™ (Lenograstim) , will be reported to Chugai Pharma UK Limited within 24 hours by fax.

9. DATA HANDLING AND RECORD KEEPING

9.1 Data Collection

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

Form	Summary of data recorded
Eligibility checklist	Check of Inclusion and Exclusion Criteria
Registration and Randomisation Form	Patient 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 Medications	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

Ad hoc forms

The majority of the data collection is done by Electronic Remote Data Capture (eRDC). For further information, please refer to the eRDC and CRF Guidelines.

Paper data collection is only used for the Serious Adverse Event form.

Serious Adverse Event form: The CRF will be completed, signed/dated by an authorised member of the site research team (as delegated on the Site Signature and Delegation Log), and co-signed by the Investigator. The CRF must be returned to the Trials Office via fax. 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 should be consistent with the source data or the discrepancies should be explained. All missing and ambiguous data will be queried and a SAE follow-up form will be required. In all cases it remains the responsibility of the Investigator to ensure that the CRF/eRDC has been completed correctly and that the data are accurate.

Trial forms may be amended by the Trials Office, as appropriate, throughout the duration of the trial. Whilst this will not constitute a protocol amendment, new versions of the form must be implemented by participating sites immediately on receipt.

9.2 Archiving

The Investigator will ensure all essential trial documentation and source records (e.g. signed Informed Consent Forms, Investigator Site Files, Pharmacy Files, patients' hospital notes, copies of CRFs etc) at their site are securely retained for at least 5 years after the end of the trial. The Trial Office will retain the original CRFs.

Regulatory authorities will have the right to audit such records in accordance with ICH GCP guidelines and EU directive 2001/20/EU.

10. QUALITY MANAGEMENT

The trial is being conducted under the auspices of the Cancer Research UK Clinical Trials Unit (CRCTU) according to the current guidelines for Good Clinical Practice (GCP). Participating sites will be monitored by CRCTU staff to confirm compliance with the protocol and the protection of participants' rights as detailed in the Declaration of Helsinki.

All participating investigators will be asked to sign the necessary agreements and supply a current CV to the Trial Office. All members of the site research team will also be required to sign the Site Signature and Delegation Log.

Prior to commencing recruitment, personnel involved in the conduct of the trial will undergo a process of initiation. This will cover trial rationale, protocol procedures and collection and reporting of data, this process will be conducted by trial staff either in person or by teleconference if appropriate. Following this an Investigator Site File will be provided containing essential documentation required for the conduct of the study. The Trial Office should be informed of any change in the site research team.

Trial staff will be in regular contact with the site research team to check on progress and address any queries they may have. Trial staff will check incoming CRFs for compliance with the protocol, data consistency, missing data and timing. All forms with missing data, anomalies or discrepancies will be returned for querying and corrections.

Sites may be suspended from further recruitment in the event of serious and persistent non-compliance with the protocol and/or ICH GCP. Any major problems will be addressed by the Trial Management Group.

On-site monitoring will be carried out as required following a risk assessment and as documented in the Quality Management Plan. If a monitoring visit is required the Trials Office will contact the site to arrange a date for the proposed visit and will provide the site with written confirmation. Investigators will allow the trial staff access to source documents as requested

11. RECRUITMENT PERIOD

Recruitment into the study is planned to take place over a 5 year period, commencing Dec 2009 and aiming to conclude Dec 2014, although recruitment will continue after this until the required number of patients are involved.

12. END OF TRIAL DEFINITION

The trial will end when the all participants have completed the required follow up.

13. STATISTICAL CONSIDERATIONS

13.1 Power Calculations

The primary outcome measure is the change in MELD score from randomisation (day 0) to 90 days post-randomisation (day 90). Analysis of 60 patients eligible for the trial in our clinic cohort demonstrated a mean baseline MELD score of 13.5, with a median change in MELD over 3 months of 0 (SD 1.0). In other words we do not expect any change in MELD over 90 days in conventionally treated patients. A clinically significant reduction would be at least a 0.8 point reduction in MELD.

The trial is designed as a three-armed study with one control arm. The trial is powered to answer two hypotheses of each treatment compared to control but not powered to detect smaller expected differences between the two treatment groups. As such, the overall α=0.1 for the trial is split equally between the two hypotheses. Conventionally, to detect a relevant standardised effect size of 0.8 point reduction in MELD score using two-sided α=0.05 (overall α=0.1 split equally between the two hypotheses) and 80% power requires 27 participants to be randomised per group (81 participants in total). The null hypothesis to be tested is that the change in mean at 90 days compared across two groups (treatment vs. control) is the same. The alternative hypothesis is that the change in mean at 90 days compared across two groups (treatment vs. control) is at least 0.8 standard deviations apart.

The number of participants lost to follow-up, or withdrawn consent prior to initial treatment is expected to be minimal. The Data Monitoring Committee (DMC) may advise replacement of participants if numbers are higher than anticipated.

13.2 Analysis of Primary Outcome Measures

Baseline MELD score will be collected at randomisation and days 30, 60 and 90 post-randomisation. Change in MELD will be calculated for each participant and average changes presented and statistically compared for each treatment group against control using 2-sample t-test or non-parametric two-sample Wilcoxon test if appropriate.

It is unknown over what period the treatment remains active or if its effect increases linearly or in some other way. As such the statistical analysis plan has been updated to include an additional co-primary final analysis entailing a repeated measures analysis over time, specifically a mixed-effect modelling procedure, taking into account the MELD measurement captured at baseline, days 30, 60 and 90. The introduction of mixed-effects modelling will make more efficient use of the patient level information, enhance the scope of the study to understand the trend of treatment activity in more detail (rather than focusing only at a specific time-point, day 90) and address missing values. To model the trend in MELD over time, a linear mixed effects models (taking into account within subject correlation) using linear, quadratic polynomials or more flexible semiparametric models will be considered. Goodness of fit tests will be used to compare the different models. We will evaluate if MELD changes over time, and if so, what is the pattern of change, as well as if the pattern differs between each treatment and control group. For this analysis the null hypothesis to be tested is that the slope of the change in MELD compared across two groups (treatment vs. control) is the same. The alternative hypothesis is that the slope of the change in MELD compared across two groups differ.

13.3 Analysis of Secondary Outcome Measures

The number of participants who do not comply with the protocol is expected to be low. All protocol violators and ineligible participants will be reported. All analyses will be carried out on a modified intention to treat basis, retaining participants in their randomised treatment groups and including protocol violator and ineligible participants, in order to maintain the unbiased comparison of treatments created by the randomisation procedure.

Changes from baseline for secondary outcomes scored as continuous measures will be calculated and presented descriptively by treatment group. As per the primary outcome, the trend in UKELD over the 90 day period will be assessed using each of the measurements captured at baseline and days 30, 60 and 90.

The proportions of participants experiencing adverse events and other clinical events will be presented descriptively by treatment group as proportions with 95% confidence intervals.

Quality of life will be analysed using longitudinal statistical methods comparing treatment groups with appropriate consideration given to missing data due to dropout and death. Questionnaire responses will be combined and transformed into dimension scores. Standardised area under the curve analysis will be used to assess mean observed symptomatic and functional QoL over a complete period of 12 months from randomisation whilst minimising multiple testing.

Survival estimates will be calculated using the method of Kaplan and Meier and presented descriptively as median, 3-month, 6-month and 12-month survival estimates with 95% confidence intervals.

13.4 Planned Subgroup Analysis

Median MELD scores will be presented descriptively across treatment groups within the stratification subgroups of aetiology of disease (ALD, HCV, Other).

13.5 Interim and Final Analysis

Reports for the DMC will be produced by the trial statistician. The first interim report will be prepared and presented to the DMC when the study has been open to recruitment for at least 12 months, or when 20 patients have been recruited to treatment groups 2+3. The aim of this report is to assess recruitment, compliance to treatment, safety data (toxicity and serious adverse events) and to assess the underlying assumptions of the power calculations. The study is expected to take 3 years to recruit and as such a second interim report is planned at the end of the second year of recruitment to additionally assess the stated outcome measures.

Final analyses will be carried out when all participants have been followed for at least one year after randomisation.

13.6 Data Management Committee

Data analyses will be supplied in confidence to an independent Data Monitoring Committee (DMC), which will be asked to give advice on whether the accumulated data from the trial, together with the results from other relevant research, justifies the continuing recruitment of further patients. The DMC will operate in accordance with a trial specific charter based upon the template created by the Damocles Group.

Additional meetings may be called if recruitment is much faster than anticipated and the DMC may, at their discretion, request to meet more frequently or to continue to meet following completion of recruitment. An emergency meeting may also be convened if a safety issue is identified.

The DMC will report directly to the Trial Management Group who will convey the findings of the DMC to the Study Sponsor, the MHRA and Ethics Committee.

The DMC may consider discontinuing the trial if the recruitment rate or data quality are unacceptable or if any issues are identified which may compromise participant safety.

14. TRIAL ORGANISATIONAL STRUCTURE

14.1 Sponsor

REALISTIC is an investigator led and designed trial, co-ordinated by the Liver Research Group within the CRCTU in Birmingham. The University of Birmingham will act as a single sponsor. The CRCTU does not hold insurance against claims for compensation for injury caused by participation in a clinical trial and they cannot offer any indemnity. As this is a clinician-initiated study, the Association of the British Pharmaceutical Industry (ABPI) guidelines for patient compensation by the pharmaceutical industry will not apply. However, in terms of liability, NHS Trust and Non-Trust Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical trial. Therefore compensation is available in the event of clinical negligence being proven.

Sponsor addresses

The University of Birmingham
Edgbaston
Birmingham
B15 2TT
United Kingdom

14.2 Finance

The trial is funded by the National Institute for Health Research (NIHR) and The Sir Jules Thorn Charitable Trust.

14.3 Trial Management Group

Membership	Chief Investigators Research Physicians Senior Trial Co-ordinator Trial Statisticians
Responsibilities	Design and Conduct of Trial Preparation of Protocol and Amendments Preparation of Patient Information Sheets and Consent Forms Preparation of CRFs Reviewing progress of Trial and if necessary agreeing changes to the protocol Providing Annual Report to MHRA and Ethics Committee SUSAR Reporting to MHRA Data Verification Data analysis Preparation of Trial Reports including DMC Reports Publication and Presentation of Results

14.4 Delegation

The Principal Investigator at each centre will be ultimately responsible for patient identification, recruitment, data collection, completion of CRFs, follow up of trial participants and adherence to study protocol.

These duties may be delegated to appropriate medical or nursing trial staff as detailed in the Site Signature and Delegation Log.

15. ETHICAL CONSIDERATIONS

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland, June 1964, amended at the 48th World Medical Association General Assembly, Somerset West, Republic of South Africa, October 1996

This trial will be carried out under a Clinical Trial Authorisation and will be conducted in accordance with the principals of Good Clinical Practice according to the EU directive 2005/28/EC (GCP Directive) and UK legislation.

The trial will be submitted to and approved by a Main Research Ethics Committee. It is the responsibility of each Principal Investigator to obtain Trust R&D approval. Sites will not be permitted to enrol patients until written confirmation of ethical and R&D approval is received by the Trials Office. It is the responsibility of the Principal Investigator to ensure that all subsequent amendments gain the necessary approval. This does not affect the individual clinicians' responsibility to take immediate action if thought necessary to protect the health and interest of individual patients.

Informed written consent will be obtained from the patients prior to inclusion in the trial. The right of a patient to refuse participation without giving reasons must be respected. The patient must be informed about their right to withdraw at any time from the trial without prejudicing their further treatment.

16. CONFIDENTIALITY AND DATA PROTECTION

The personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the 1998 Data Protection Act. Patients will be identified using only their unique trial number, initials, hospital number and date of birth on all CRFs and any correspondence between the Study Office and the participating site.

The Investigator must maintain documents not for submission to the Trial Office in strict confidence. In the case of special problems and/or governmental queries, it will be necessary to have access to the complete study records, provided that patient confidentiality is protected.

The CRCTU at The University of Birmingham will maintain the confidentiality of all patient data and will not disclose information by which patients may be identified to any third party, other than those directly involved in the treatment of the patient's cancer. Representatives of the trial team may be required to have access to patient notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times.

17. INSURANCE AND INDEMNITY

The University of Birmingham will act as single sponsor. The Cancer Research Clinical Trials Unit, Birmingham will coordinate the study on behalf of the Sponsors.

In terms of liability, NHS Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical trial. Compensation is only available in the event of clinical negligence being proven. There are no specific arrangements for compensation made in respect of any serious adverse events occurring through participation in the trial, whether from side effects listed, or others yet unforeseen.

University of Birmingham employees are indemnified by the University insurers for negligent harm caused by the design or co-ordination of the clinical trials they undertake while in the University's employment.

18. PUBLICATION POLICY

Results of this trial will be submitted for publication in a peer reviewed journal. The manuscript will be prepared by the Trial Management Group (TMG) and authorship will be determined by mutual agreement.

19. REFERENCE LIST

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APPENDIX 1 - 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 patients, 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 patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient'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 patients 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 patients, 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 patients 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 patients 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, patients 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 patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient 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 2- CHRONIC LIVER DISEASE QUESTIONNAIRE

Patient Number		Patient TNO		
Date of Questionnaire	D D	M O N	Y Y Y Y	
Assessment (circle):	Day 1 Screening	Day 90 Visit 5	Day 180 Visit 6	Day 360 Visit 7

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 last 2 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 2 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