Safety profile of autologous macrophage therapy for liver cirrhosis

1. Extended Data

Complete the Inventory below for all Extended Data figures.

Figure #	Figure title One sentence only	Filename This should be the name the file is saved as when it is uploaded to our system. Please include the file extension. i.e.: *Smith_ED Fig1.jpg*	Figure Legend If you are citing a reference for the first time in these legends, please include all new references in the Online Methods References section, and carry on the numbering from the main References section of the paper.
Extended Data Fig. 1	Representative flow cytometry analysis from macrophage manufacturing process.	Fobes_ED Fig1.jpeg	Samples analysed using a BD FACS Canto II flow cytometer. a) Leukapheresis start material from a patient enrolled in the trial before and after CliniMACS prodigy selection of CD14+ cells. Samples gated on live, singlet, CD45+ cells as described in Fraser et al. Cytotherapy 2017;19:1113-24. Pre-selection, leukapheresis material contains a population of CD14-high mononuclear cells, which is enriched to >95% after CliniMACS Prodigy Selection. b) Enriched macrophages at day 0 and after 7 days of culture in Macrophage-Colony Stimulating Factor (M-CSF). Fewer than 3% of CD14+ cells express the macrophage marker 25FP, which has risen to more than 86% after 7 days culture. Samples gated on live, singlet, CD45+ cells as described in Fraser et al. Cytotherapy 2017;19:1113-24. The product meets the specification of > 80% live CD45+ 25F9+ cells with a delta mean fluorescence change in 25F9 expression of >5x versus the start material as discussed in Fraser et al. Cytotherapy 2017;19:1113-24. (Actual delta 25F9 mean fluorescence is 6.85 in this case).
Extended Data Fig. 2	Dose-limiting toxicity, by dose of cells infused, expressed as change from baseline over time.	Forbes_ED Fig2.jpeg	DLT = dose-limiting toxicity. a) Fold-change in serum alanine aminotransferase (ALT); DLT defined as >3-fold. b) Fold-change in serum total bilirubin; DLT defined as >3-fold. c) Fold-change in serum creatinine; DLT defined as ≥1.5-fold. d) Fold-change in haemoglobin; DLT defined as >-1.5 fold. One subject in 10^7 cell dose group developed anaemia at

			360-day follow-up visit. This was confirmed, after the trial was completed, to be related to florid portal hypertensive gastropathy. e) Fold-change in platelets; DLT defined as >-2 fold. f) Total white cells count absolute numbers; DLT defined as < 2.0 x109/µL.
Extended Data Fig. 3	Selected safety-related serum cytokine levels, by dose of cell infused, expressed as change from baseline over time.	Forbes_ED Fig3.jpeg	All cytokine measurements are in pg/mL. a) Changes in IL8 levels from baseline. b) Changes in IL1□ from baseline – two subjects in dose group 108 cells had undetectable IL1□ levels. c) Changes in IL6 from baseline. d) Changes in TNF□ from baseline. e) Changes in INFγ from baseline. f) Changes in IL10 changes baseline.
Extended Data Fig. 4	Change in MELD score from baseline over time and in the first month after cell infusion.	Forbes_ED Fig4.jpeg	a) Individual participant data, classified by cell dose group (n=3 per group), expressed as the delta-MELD from baseline (dotted black line) over time. Time-points indicate the time of macrophage infusion (black line; approximately 14 days from baseline) and study-specific follow-up visits in the trial. Primary and secondary outcomes were measured at day-90 post-infusion. b) Individual participant data by cell dose expressed over initial safety and follow-up visits up to 30 days after infusion of macrophages (indicating MELD changes closer to infusion time-point).
Extended Data Fig. 5	Assessments of liver function, by dose of infused cells, expressed as changes from baseline over time.	Forbes_ED Fig5.jpeg	a) Changes in United Kingdom End-Stage Liver Disease (UKELD) score from baseline (arbitrary units). b) Changes in serum albumin (g/dL) from baseline.
Extended Data Fig. 6	Transient elastography (Fibroscan®) results (kPa), by dose of infused cells, expressed as changes from baseline over time.	Forbes_ED Fig6.jpeg	One-dimensional transient elastography was performed in fasted subjects using FibroScan® (Echosens, Paris, France) by fully trained and certified operators, using either an M or XL probe to obtain ten valid readings, with a success rate of at least 60% and IQR <30% of the median result. Three results did not meet the manufacturer's recommended validity criteria and were therefore removed (baseline measure for participant 004 and participant 005 and 90

			days measure for participant 008).
Extended Data Fig. 7	Assessment of non-invasive serum liver fibrosis markers (individual Enhanced Liver Fibrosis (ELF) test components), by dose of infused cells, expressed as changes from baseline over time.	Forbes_ED Fig7.jpeg	a) Changes in serum hyaluronic acid (ng/mL) from baseline. b) Changes in serum procollagen III amino terminal peptide (PIIINP; ng/mL) from baseline. c) Changes in serum tissue inhibitor of metalloproteinase 1 (TIMP-1; ng/mL) from baseline.
Extended Data Fig. 8	Measurement of health-related quality of life scores using the Chronic Liver Disease Questionnaire (CLDQ) instrument, by dose of cells infused, expressed as change from baseline over time.	Forbes_ED Fig8.jpg	Measurement of health-related quality of life scores using the Chronic Liver Disease Questionnaire (CLDQ) instrument, by dose of cells infused, expressed as change from baseline over time. CLDQ domains are assessed using seven-point scales, ranging from the worst (1) to the best (7) possible function. a) Changes in "Emotional" domain score from baseline. b) Changes in "Worry" domain score from baseline. Each line in each of the graphs represents data from an individual participant.

Delete rows as needed to accommodate the number of figures (10 is the maximum allowed).

2. Supplementary Information:

A. Flat Files

Complete the Inventory below for all additional textual information and any additional Supplementary Figures, which should be supplied in one combined PDF file.

Item	Present?	Filename	A brief, numerical description of file

		This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. The extension must be .pdf	contents. i.e.: *Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.*
Supplementary Information	yes	Forbes supplementary information complete.pdf	Supplementary Table 1, Original study protocol for phase 1 MATCH study
Reporting Summary	yes	Forbes_reporting summay.pdf	

B. Additional Supplementary Files

Complete the Inventory below for all additional Supplementary Files that cannot be submitted as part of the Combined PDF.

Type	Number If there are multiple files of the same type this should be the numerical indicator. i.e. "1" for Video 1, "2" for Video 2, etc.	Filename This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: *Smith_ Supplementary Video 1.mov*	Legend or Descriptive Caption Describe the contents of the file
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Source Data Fig. 1	Forbes_source data Fig2.xls	Statistical source data
Source Data Fig. 2	Forbes_source data Fig3.xls	Statistical source data
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Safety profile of autologous macrophage therapy for liver cirrhosis

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ABSTRACT

Therapies to reduce liver fibrosis and stimulate organ regeneration are urgently needed. We conducted a first-in-human, phase 1 dose-escalation trial of autologous macrophage therapy in 9 adults with cirrhosis and Model for End-Stage Liver Disease (MELD) score of 10-16 (ISRCTN10368050). Groups of 3 participants received a single peripheral infusion of 10^7, 10^8, or up to 10^9 cells. Leukapheresis and macrophage infusion was well-tolerated with no transfusion reactions, dose-limiting toxicities or macrophage activation syndrome. All participants were alive and transplant-free at 1 year, with only 1 clinical event recorded, the occurrence of minimal ascites. The primary outcomes of safety and feasibility were met. This study informs and provides a rationale for efficacy studies in cirrhosis and other fibrotic diseases.

INTRODUCTION

Globally, liver cirrhosis currently causes 1.16 million deaths every year. In the US, among people aged 45–64 years, chronic liver disease is the 4th leading cause of death.[1] Cause-specific interventions are effective, but patients often present with advanced liver disease and cirrhosis. No curative options are available for cirrhosis except for organ transplantation which requires major surgery and lifelong immunosuppression. Donor organ availability also restricts access to transplantation.[2] Alternative therapies to treat cirrhosis are therefore being developed including cell therapies.[3,4]

The macrophage is a cellular regulator of liver fibrosis deposition and resolution.[5] During disease progression macrophages release signals which drive inflammatory cell recruitment and activation of hepatic stellate cells to produce extracellular matrix (ECM). Following cessation of injury, macrophages release matrix metalloproteinases (MMPs) that promote fibrotic ECM degradation, and factors that dampen the inflammatory response[6-8,9] and drive liver regeneration.[7,10]

In mouse models of liver fibrosis, macrophages injected via a peripheral vein home to the liver, express MMPs, and recruit host immune cells to liver scar via chemokine expression, ameliorating liver fibrosis, stimulating liver regeneration and improving function.[10] Circulating $CD14^+$ monocytes

can be isolated from cirrhotic patient mononuclear cell (MNC) leukapheresis products with high yield and purity and can be differentiated using Good Manufacturing Practice (GMP)-compliant processes into macrophages with a comparable phenotype to those from healthy volunteers.[11,12] These macrophages can also resolve liver fibrosis in mouse models.[12] These data prompted us to conduct a first-in-human, phase 1, single-arm, dose-escalation clinical trial in people with cirrhosis evaluating maximum-tolerated dose and safety of peripheral infusion of *ex vivo* matured autologous monocyte-derived macrophages.

RESULTS

Trial population, baseline and treatment characteristics

11 participants (4 female and 7 male, mean age 58.54±5.85) with compensated liver cirrhosis and MELD score between 10 and 16 attended a single centre (Royal Infirmary of Edinburgh, UK) for screening between 08 August 2016 and 27 March 2017 (Fig. 1). Two individuals did not meet screening criteria. Nine participants were enrolled in the trial and were followed-up for 1 year to 06 April 2018. Demographic and baseline characteristics of study participants are shown in Table 1. The mean duration of cirrhosis was 5.22±4.22 years. All participants were abstinent from alcohol at the time of recruitment except for one individual who had a history of intermittent low-level alcohol consumption (1-10 units per week). A week before the planned treatment, participants underwent a standard leukapheresis to collect circulating monocytes. Monocytes were isolated from MNC and the Investigational Medical Product (IMP) produced in a licensed GMP manufacturing facility (Extended Data 1).

Each group of 3 participants (9 in total) received a single infusion of autologous macrophages at 10^7, 10^8 or up to 10^9 cells, respectively in a dose-escalation manner. All participants were successfully evaluated for safety, feasibility and maximum-achieved safe dose of autologous macrophages. We also measured changes in: markers of liver fibrosis (serum Enhanced Liver Fibrosis (ELF™) test (Siemens Healthineers, UK), serum PRO-C3 and C3M (Nordic Bioscience, Denmark) and transient elastography (Fibroscan®, Echosens, France)); liver function (MELD and

UKELD scores); health-related quality of life (HRQL) using the Chronic Liver Disease Questionnaire (CLDQ) instrument; transplant-free survival and number of clinical events related to decompensation of cirrhosis.

Safety outcomes

All participants completed 1-year of follow-up after macrophage infusion. No participants withdrew from the study and none developed acute transfusion reactions during macrophage infusion or in the 12h post-infusion observation period. A total of 3 serious adverse events were recorded; these were assessed as mild in severity, unrelated to the IMP and there were no sequelae (Table 2). There were 70 adverse events documented in the reporting period (Table 2). A single clinical event occurred, described as a small volume of ascites around the liver on ultrasound. 9/22 (41%), 8/19 (42%) and 6/29 (21%) adverse events were considered possibly related to the IMP in the 10^7, 10^8 and up to 10^9 cell dose groups, respectively. Overall, 56% of adverse events were considered unrelated to the IMP. No dose-toxicity relationships were identified. At the end of the study period all 9 participants were alive and transplant-free.

Serum ALT and bilirubin changes at 90-days were respectively 0.88±0.21 and 0.80±0.30-fold from baseline. Fluctuation in platelet count is common in patients with cirrhosis and portal hypertension, but we did not observe a reduction in platelets to lower than 30% from baseline or clinically significant thrombocytopenia. The baseline total white cell count varied in this study population. As expected, total circulating leukocyte counts were affected by leukapheresis, but returned to baseline prior to infusion (7 days after leukapheresis). In some individuals we noted a small and transient increase in white cell count following infusion of macrophages which did not persist beyond 7 days post-infusion (Extended Data 2). Serum cytokines (including IL1α, IL6, IL8, IL10, TNFα and IFNγ) did not change significantly from baseline (Extended Data 3). Specifically, levels of IL8 (which correlate with risk of macrophage activation syndrome (MAS)) decreased transiently after macrophage infusion, with a delta of -8.23±14.39 pg/mL at 30 days and of -1.58±13.54 pg/mL at 90 days.

Secondary outcomes

At day 90 following macrophage infusion, six out of 9 participants showed a decrease in MELD score (Fig. 2 and Extended Data 4). For all patients, the MELD at baseline was 11.88±1.40 (range 9.90 to 13.87) with a mean Δ-MELD at 90 days of -1.12±1.87 (range -4.90 to 1.76). (Fig. 2 and Extended data 4). At 1-year follow-up MELD decreased in 7 out of 9 participants; with a mean Δ-MELD for all patients at 1 year of -0.910±1.24 (range -2.41 to 1.68). Overall, we did not observe a clear dose-related response; however, in the highest cell group the MELD scores all followed a similar downward trajectory over the period of follow up (Fig. 2). The mean Δ-UKELD score for all participants at 90 days was -0.42±2.27. Serum albumin levels at 90 days showed little change from baseline in all participants with mean Δ-albumin of -0.20±.0.23 g/dL, with range +0.2 to -0.5 (Extended Data 5). Similarly, INR was unaffected in all participants by macrophage infusion, with mean ±SD change from baseline of -0.04±0.09 and -0.06±0.09 at 90 days and 360 days respectively.

To detect a change in fibrosis, a range of non-invasive markers of liver fibrosis were quantified. The technical success rate of transient elastography was 91.66%. Data not meeting the quality specification as per manufacturer recommendation were removed (2 baseline and 1 90-day measurements). Baseline liver stiffness measurements were consistent with cirrhosis (mean 57.44±24.01 kPa). In 5 out of 9 participants liver stiffness measurements decreased by >6 kPa at 1-year of follow-up, with an overall mean reduction of -11.91±10.55 kPa (Extended Data 6). While a change of 6 kPa might be considered meaningful in the context of pre-cirrhotic liver fibrosis,[13] the importance of this change in established cirrhosis is uncertain. There was a downward trend in ELF scores following macrophage infusion (Fig. 3a). The mean ELF score at baseline was 12.43±0.94 with mean delta-ELF at 90 days of -0.24±0.46 and at 1 year of -1.13±1.21 (Extended Data 7). There was a similar change in serological markers of type-III collagen turnover, with mean % change of PRO-C3 of -14.86±14.50 and % change of C3M of -10.95±13.37 ng/mL at day 90 (Fig. 3b-c). The larger % decrease in PRO-C3 could indicate a predominant decrease in fibrogenic activity following infusion of macrophages. Longitudinal of health-related quality of life scores (HRQL) assessment showed relatively small variations in composite Chronic Liver Disease

Questionnaire (CLDQ) scores over time, but 5 out of 9 participants showed an improvement in overall HRQL at day 90 post-macrophage infusion (Fig. 3d and Extended Data 8). Individual domain scores are shown in Extended Data Table 1.

DISCUSSION

This first-in-human trial confirmed the safety and feasibility of a single peripheral infusion of autologous macrophages in participants with compensated liver cirrhosis of differing aetiology. Leukapheresis was well-tolerated by all participants with minimal side effects. Administration of macrophages was safe, with no clinically relevant adverse reactions recorded during the infusion or in the immediate post-infusion period. The 3+3 trial dose-escalation model is designed to define a maximum-tolerated dose. Due to monocyte isolation and macrophage production limitations, we were able to generate a "maximum-achieved dose" of up to 10^9 cells (specifically 0.8 x 10^9 cells), for which we sought to determine the safety and feasibility.

As expected, in a study population with advanced cirrhosis and other co-morbidities, we observed adverse events throughout the study. One participant had a previous history of intermittent low-level alcohol consumption, but serial gamma-glutamyl transpeptidase (GGT) levels (a biochemical marker of alcohol consumption) remained static at all follow-up visits, suggesting that this did not influence the measured outcomes for this patient. Most of the adverse events recorded in the study were exacerbations of existing conditions or minor self-limiting events. The 3 serious adverse events were considered mild and unrelated to the IMP. Among AEs possibly related to the IMP, none had Common Terminology Criteria for Adverse Events (CTCAE) severity grading over 2. There were no dose-related phenomena. All participants reached 360 days of follow-up and were transplant-free. We listed a single clinical event (worsening ascites) during the whole follow-up period. This was identified on ultrasound and resolved with diuretics. All other participants remained well compensated.

Although we did not label the infused macrophages, previous animal models and human case reports[14] suggest that macrophages infused via peripheral or central veins will transiently pass through the lungs, before engrafting in the liver and spleen.[10,15,16] While this does not prove that the

cell product used in our study reached the liver, these observations are supportive. We did not record any clinically meaningful changes in respiratory rate or oxygen saturation at any point during infusion or 12-hour follow-up period. Overall the IMP appeared safe during administration and the extended follow-up period of 360 days.

This single-arm phase 1 study was not designed or powered to demonstrate statistically significant changes in efficacy measures following macrophage therapy. However, in 6 of 9 participants reductions in MELD score were observed at 90 days, largely due to a decrease in serum bilirubin. This contrasts with a recent RCT using autologous CD133+ stem cells in adults with cirrhosis of comparable severity to this study which showed no improvement in MELD score.[17] In one individual, total bilirubin and MELD score were higher at 360 days of follow-up compared to baseline; however, over 85% of the total bilirubin was unconjugated, representing haemolysis likely due to cold agglutinins (the patient had treated hepatitis C with sustained viral response). Other parameters of liver function did not change in response to cell infusion, including UKELD score and serum albumin. Overall, no robust dose-dependent treatment effects were observed in secondary outcomes.

The macrophages manufactured using GMP-compliant processes have been comprehensively characterised and demonstrate a mature phenotype (CD14+ / high 25F9 expression), plus retention of high levels of markers associated with tissue repair and inflammation resolution (CD206, CD163 and CD169).[11]

A number of non-invasive measures of liver fibrosis improved following macrophage infusion including transient elastography, serum ELF score and the collagen turnover markers PRO-C3 and C3M, highlighting the potential antifibrotic effect of autologous monocyte-derived macrophage infusion in cirrhosis.

There was variability in measured responses to macrophage infusion, even in participants treated with the same cell dose. This likely reflects the multiple factors that could determine the effect of macrophage infusion in an individual with cirrhosis such as duration and aetiology of liver disease, other comorbidities, or engraftment and survival of the infused macrophages in the liver. The influence of these variables will be better addressed in a larger randomised controlled phase 2 trial.

Impairment of HRQL is reported by most patients with advanced cirrhosis and HRQL scores improve significantly following liver transplantation.[18] Given that a change of 0.5 on the 1 to 7 scale represents an important difference in CLDQ score, 5 of 9 participants exhibited an improvement in overall HRQL score at day 90 post-infusion.[19] In the remaining participants, composite CLDQ scores were either unchanged (n=2) or worse (n=2) at 90 days. Interestingly, there was an improvement in most participants in the emotional domain at day 90 post-infusion. We noted an inverse association between delta-MELD and CLDQ scores. Moreover, in the 4 individuals in whom MELD failed to decrease or worsened, we observed no improvement in HRQL. [19]

This first-in-human study confirmed the safety, feasibility and maximum-achievable dose of autologous macrophages and facilitate future efficacy studies in cirrhosis and other fibrotic diseases. The effects of macrophage therapy upon efficacy measures including transplant-free survival, MELD and UKELD score, fibrosis markers and HRQL will be evaluated in an ongoing phase 2 randomised controlled trial (ISRCTN 10368050).

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	Screen Failure (n=2)		10^7 Cells (n=3)			10^8 Cells (n=3)			Up to 10^9 Cells (n=3)		
									$0.6x10^9$	$0.8x10^9$	$0.7x10^9$
Participant ID	*001*	*002*	*003*	*004*	*005*	*006*	*007*	*008*	*009*	*010*	*011*
DEMOGRAPHICS											
Mean Age (+/-SD)	63.00 ±5.66		59.33 ±8.50			55.67 ±6.35			57.67± 2.88		
Body Mass Index	32.1	28.2	24.7	29.6	35.6	26	27.8	27.8	33.6	27.6	29
Sex (Male:Female)	2:0		1:2			3:0			1:2		
Ethnicity	All Caucasian		All Caucasian			All Caucasian			All Caucasian		
AETIOLOGY OF LIVER DISEASE											
ALD (n)	1		2			2			2		
NAFLD (n)	1		0			0			1		
HCV (SVR) (n)	0		0			1			0		
PBC (n)	0		1			0			0		
SEVERITY OF CIRRHOSIS											
MELD score			13	11	14	13	10	13	10	13	11
Mean MELD score (+/-SD).			12.37±1.51			11.90±1.48			11.36±1.62		
UKELD score			50	50	50	51	51	51	48	51	47
Child-Pugh score			6	5	7	6	6	8	5	9	9
Child-Pugh class			A	A	B	A	A	B	A	B	B
LIVER DISEASE COMPLICATIONS											
Ascites	x		x				x	x		x	x
SBP											
Variceal bleeding			x				x	x		x	x
Hepatic encephalopathy										x	x

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Table 1. Baseline characteristics of trial participants classified by cell dose group. ALD, alcohol-related liver disease; NAFLD, non-alcoholic fatty liver disease; HCV, hepatitis C virus; SVR, sustained viral response (> 6 months); PBC, primary biliary cholangitis; MELD, Model for End-Stage Liver Disease; UKELD, United Kingdom Model for End-Stage Liver Disease; SBP, spontaneous bacterial peritonitis. Measures of error for mean age and MELD are standard deviation (SD).

Adverse Event	**10^7 cell dose**	**10^8 cell dose**	**Up to 10^9 cell dose**
Nausea	1	0	0
Abdominal pain	0	2	3
Anorexia	0	1	0
Light-headedness	1	2	2
Fatigue	1	1	3
Chest pain	4	6	0
Joint pain/malaise	2	2	3
Rash	2	0	3
Hypocalcaemia symptoms (leukapheresis)	1	2	3
Ascites	0	1	0
Anaemia	1	1	0
Infective	3	0	2
Others	5	1	10
TOTAL	**22**	**19**	**29**
Number of probably related AEs	**9 (41%)**	**8 (42%)**	**6 (21%)**
Type of Serious Adverse Event			
Abdominal pain and constipation			2
Papillary lesion of breast	1		

Table 2. Recorded adverse events and serious adverse events during the study period. Adverse events (AEs) and serious adverse events (SAEs) classified by dose, using Medical Dictionary for Regulatory Activities (MedDRA) coding version 20.0. All AEs listed were defined as grade 1 or 2 according to the Common Terminology Criteria for Adverse Events version 5.0. All the SAE were considered unrelated to the macrophage infusion. Two, although rated of mild severity,

resulted in overnight admission to hospital. The SAE relative to the incidental finding of a papillary lesion of breast through screening mammogram led to surgical excision

Fig. 1. Trial profile**.** A 3+3 model for dose escalation was used. During the study, there was no dose-limiting toxicity (DLT); therefore, only 9 participants were needed to complete the dose-escalation phase.

Fig. 2. MELD score over time per cell dose group. Each line represents a participant in the trial. Time-points indicate the time of macrophage infusion (purple line; approximately 14 days from baseline) and study-specific follow-up visits in the trial. Primary and secondary outcomes were measured at day-90 post-infusion. **a)** 10^7 cells; **b)** 10^8 cells; **c)** up to 10^9 cells.

Fig. 3. Secondary outcomes a) Individual participant ELF score changes from baseline (BL) over time (delta-ELF). **b)** Individual participant PRO-C3 level changes from baseline over time (% changes of PRO-C3). **c)** Individual participant C3M level changes from baseline over time (% changes of C3M). **d**) Individual self-reported health related quality of life (HRQL) measures over time, expressed as the composite Chronic Liver Disease Questionnaire (CLDQ) score and not delta changes to highlight the significant variability in baseline HRQL composite score in this population. All data are shown by dose group (n=3).

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Author Contributions

Conceptualization and design of the work were carried out by S.J.F., C.P., L.R., L.B., D.M., A.L., S.D., E.H., A.R.F., M.L.T., J.D.M.C., N.W.A.M., J.B., J.K.M., P.C.H., J.A.F.; the acquisition, analysis, and interpretation of data were performed by S.J.F., J.A.F., F.M., B.D., C.G., D.J.L., M.J.N., K.M.; trial delivery and administration were carried out by F.M., A.G.; the original draft of the manuscript was written by F.M.; the draft was reviewed and edited by all the authors.

Competing interests

J.A.F. reports personal fees from Novartis, Ferring Pharmaceuticals, Galecto Biotech, Caldan Therapeutics, Gilde Healthcare, Arix Bioscience, Guidepoint and grants from GlaxoSmithKline, Novartis and Intercept Pharmaceuticals, outside the submitted work. S.J.F. has a grant from Syncona to develop macrophages as a therapy. D.J.L., K.M., M.J.N. are full-time employees at Nordic Bioscience. D.J.L., M.K. and M.J.N. are among the original inventors and patent holders of C3M and PRO-C3. D.J.L. holds stock in Nordic Bioscience. P.C.H. is an advisor for AbbVie, BMS, Eisai Ltd, Falk, Ferring, Gilead, Gore, Janssen, Lundbeck, MSD, Norgine, Novartis, ONO Pharmaceuticals, Pfizer and Roche, outside the submitted work.

REFERENCES

1. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. Journal of hepatology 2019;70:151-71.
2. D'Amico G, Garcia-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. Journal of hepatology 2006;44:217-31.
3. Forbes SJ, Gupta S, Dhawan A. Cell therapy for liver disease: From liver transplantation to cell factory. J Hepatol 2015;62:S157-69.
4. Schuppan D, Kim YO. Evolving therapies for liver fibrosis. The Journal of clinical investigation 2013;123:1887-901.

5. Duffield JS, Forbes SJ, Constandinou CM, et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 2005;115:56-65.
6. Gouw AS, Clouston AD, Theise ND. Ductular reactions in human liver: diversity at the interface. Hepatology (Baltimore, Md) 2011;54:1853-63.
7. Boulter L, Govaere O, Bird TG, et al. Macrophage-derived Wnt opposes Notch signaling to specify hepatic progenitor cell fate in chronic liver disease. Nat Med 2012;18:572-9.
8. Ramachandran P, Pellicoro A, Vernon MA, et al. Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. Proc Natl Acad Sci U S A 2012;109:E3186-95.
9. Fallowfield JA, Mizuno M, Kendall TJ, et al. Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. Journal of immunology (Baltimore, Md : 1950) 2007;178:5288-95.
10. Bird TG, Lu WY, Boulter L, et al. Bone marrow injection stimulates hepatic ductular reactions in the absence of injury via macrophage-mediated TWEAK signaling. Proc Natl Acad Sci U S A 2013;110:6542-7.
11. Fraser AR, Pass C, Burgoyne P, et al. Development, functional characterization and validation of methodology for GMP-compliant manufacture of phagocytic macrophages: A novel cellular therapeutic for liver cirrhosis. Cytotherapy 2017;19:1113-24.
12. Moore JK, Mackinnon AC, Wojtacha D, et al. Phenotypic and functional characterization of macrophages with therapeutic potential generated from human cirrhotic monocytes in a cohort study. Cytotherapy 2015;17:1604-16.
13. Foucher J, Chanteloup E, Vergniol J, et al. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. Gut 2006;55:403-8.
14. Hutchinson JA, Riquelme P, Sawitzki B, et al. Cutting Edge: Immunological consequences and trafficking of human regulatory macrophages administered to renal transplant recipients. Journal of immunology (Baltimore, Md : 1950) 2011;187:2072-8.
15. Sharkey J, Starkey Lewis PJ, Barrow M, et al. Functionalized superparamagnetic iron oxide nanoparticles provide highly efficient iron-labeling in macrophages for magnetic resonance-based detection in vivo. Cytotherapy 2017;19:555-69.
16. Thomas JA, Pope C, Wojtacha D, et al. Macrophage therapy for murine liver fibrosis recruits host effector cells improving fibrosis, regeneration, and function. Hepatology 2011;53:2003-15.
17. Newsome PN, Fox R, King AL, et al. Granulocyte colony-stimulating factor and autologous CD133-positive stem-cell therapy in liver cirrhosis (REALISTIC): an open-label, randomised, controlled phase 2 trial. Lancet Gastroenterol Hepatol 2018;3:25-36.
18. Younossi ZM, McCormick M, Price LL, et al. Impact of liver transplantation on health-related quality of life. Liver transplantation : official publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society 2000;6:779-83.
19. Younossi ZM, Guyatt G, Kiwi M, Boparai N, King D. Development of a disease specific questionnaire to measure health related quality of life in patients with chronic liver disease. Gut 1999;45:295-300.
20. Le Tourneau C, Lee JJ, Siu LL. Dose escalation methods in phase I cancer clinical trials. Journal of the National Cancer Institute 2009;101:708-20.
21. Kullak-Ublick GA, Andrade RJ, Merz M, et al. Drug-induced liver injury: recent advances in diagnosis and risk assessment. Gut 2017;66:1154-64.
22. Mehta RL, Kellum JA, Shah SV, et al. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. Crit Care 2007;11:R31.
23. Henter JI, Horne A, Arico M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48:124-31.
24. Tinegate H, Birchall J, Gray A, et al. Guideline on the investigation and management of acute transfusion reactions. Prepared by the BCSH Blood Transfusion Task Force. British journal of haematology 2012;159:143-53.
25. Day J PP, Parkes J, et al. Derivation and Performance of Standardized Enhanced Liver Fibrosis (ELF) Test Thresholds for the Detection and Prognosis of Liver Fibrosis. . the journal of applied laboratory medicine 2018;3.

26. Friedrich-Rust M, Rosenberg W, Parkes J, Herrmann E, Zeuzem S, Sarrazin C. Comparison of ELF, FibroTest and FibroScan for the non-invasive assessment of liver fibrosis. BMC gastroenterology 2010;10:103.
27. Karsdal MA, Nielsen MJ, Sand JM, et al. Extracellular matrix remodeling: the common denominator in connective tissue diseases. Possibilities for evaluation and current understanding of the matrix as more than a passive architecture, but a key player in tissue failure. Assay and drug development technologies 2013;11:70-92.
28. Nielsen MJ, Nedergaard AF, Sun S, et al. The neo-epitope specific PRO-C3 ELISA measures true formation of type III collagen associated with liver and muscle parameters. American journal of translational research 2013;5:303-15.
29. Veidal SS, Vassiliadis E, Barascuk N, et al. Matrix metalloproteinase-9-mediated type III collagen degradation as a novel serological biochemical marker for liver fibrogenesis. Liver international : official journal of the International Association for the Study of the Liver 2010;30:1293-304.
30. Leeming DJ, Veidal SS, Karsdal MA, et al. Pro-C5, a marker of true type V collagen formation and fibrillation, correlates with portal hypertension in patients with alcoholic cirrhosis. Scand J Gastroenterol 2015;50:584-92.
31. Karsdal MA, Hjuler ST, Luo Y, et al. Assessment of liver fibrosis progression and regression by a serological collagen turnover profile. American journal of physiology Gastrointestinal and liver physiology 2019;316:G25-g31.
32. Huo TI, Wu JC, Lin HC, et al. Evaluation of the increase in model for end-stage liver disease (DeltaMELD) score over time as a prognostic predictor in patients with advanced cirrhosis: risk factor analysis and comparison with initial MELD and Child-Turcotte-Pugh score. Journal of hepatology 2005;42:826-32.
33. Barber K, Madden S, Allen J, Collett D, Neuberger J, Gimson A. Elective liver transplant list mortality: development of a United Kingdom end-stage liver disease score. Transplantation 2011;92:469-76.
34. Loria A, Escheik C, Gerber NL, Younossi ZM. Quality of life in cirrhosis. Current gastroenterology reports 2013;15:301.

METHODS

Study oversight

The MATCH 0.1 trial is an investigator-led study, funded by the Medical Research Council (Reference: MR/M007588/1) and sponsored by ACCORD (Academic and Clinical Central Office for Research and Development for NHS Lothian/University of Edinburgh). All study-related documents were designed by the trial team with input from ACCORD, an independent statistician and the Scottish National Blood Transfusion Service (SNBTS) team. The trial was approved by Scotland A Research Ethics Committee (Reference: 15/SS/0121), NHS Lothian Research and Development department and the Medicine and Health Care Regulatory Agency (MHRA-UK). The trial was registered in the International Standard Randomized Controlled Trial registry (ISRCTN10368050) and the European Clinical Trial Database (Reference: 2015-000963-15). All participants enrolled in the study gave informed consent and the trial was conducted under Good Clinical Practice regulations.

Study design

A phase 1 first-in-human trial using a standard 3+3 dose-escalation design was conducted in a single centre (Royal Infirmary of Edinburgh, Edinburgh, UK).[20] Due to limitations in production and cell selection, the maximum number of cells that could be produced for infusion was 10^9; this study was therefore designed to ascertain the tolerability of the maximum-achievable dose and not the maximum-tolerated dose. This approach was approved by the appropriate oversight bodies (Phase I/First in Human Study Review Committee, Data Monitoring Committee and Trial Steering Committee). Escalation decisions were taken by an independent Data Monitoring Committee and recommendations discussed within the Trial Steering Committee and acted upon before each dose-escalation.

Study participants

All participants were recruited through the hospital outpatient service in NHS Lothian between 08 August 2016 and 06 April 2018. 9 adult participants with liver cirrhosis of different aetiologies and a MELD score between 10 and 16 were enrolled. To confirm eligibility only, we used a MELD

calculator adopted by the transplant coordinators within our unit; this rounds MELD score to the nearest integer. Full inclusion and exclusion criteria are detailed in the protocol in the Extended Data. Inclusion criteria included: age 18-75; MELD score 10-16 (inclusive); liver disease aetiology of alcohol-related liver disease, primary biliary cholangitis, non-alcoholic fatty liver disease, cryptogenic cirrhosis, haemochromatosis, alpha-1 antitrypsin deficiency or treated chronic hepatitis C (sustained viral response); liver cirrhosis (diagnosed by at least one of: liver biopsy, Fibroscan™ median liver stiffness measurement >15 kPa, or clinical and radiological evidence consistent with cirrhosis). Exclusion criteria included: history of decompensated cirrhosis in the previous 3 months (portal hypertensive bleeding, ascites requiring medical treatment or hepatic encephalopathy requiring hospitalisation); hepatocellular carcinoma or undetermined liver nodules; cancer in the previous 5 years (excluding adequately treated and localised skin cancer or carcinoma-in-situ of the cervix); previous organ or tissue transplantation; listed for liver transplant; pregnancy and breastfeeding; presence of acute illness that may compromise safety of the patient in the trial. No active alcohol misuse ≥6 calendar months prior to screening was permitted. Individuals attended for a screening visit to ensure eligibility 7±4 days before scheduled leukapheresis. Participants underwent leukapheresis a week before infusions. The Investigational Medical Product (IMP) was produced in a GMP-accredited facility. On the day of infusion, active infection was excluded by physical examination and laboratory investigations. Prior to infusion, 10 mg i.v. chlorphenamine and 100 mg i.v. hydrocortisone was administered. Each group of 3 participants received a single infusion given over 30 +/- 5 minutes of 10^7, 10^8 and up to 10^9 cells, respectively.

Study Assessments

During infusion, participants were monitored closely and observed overnight in the RIE Clinical Research Facility (CRF). Special arrangements were in place with the intensive care unit in the event of a severe reaction. The following morning full blood count, renal function, electrolytes, liver function tests, triglycerides and ferritin were checked prior to discharge to exclude toxicity, including Macrophage Activation Syndrome (MAS).

During the first two follow-up visits (day 7 and day 14 after IMP infusion) safety, dose-limiting toxicity (DLT) and the presence of MAS were assessed. The definition of DLT was formulated

using accepted criteria:[21-24] serum creatinine ≥ 1.5-fold from baseline, haemoglobin 1.5-fold ≤ baseline, platelets < 2-fold from baseline, total white cell count < 2.0 x 10^9, alanine aminotransferase (ALT) > 3-fold from baseline, total bilirubin > 3-fold from baseline, MELD score > 4 points from baseline. Thereafter, participants were followed up at day 30, 60, 90, 180 and 360 after IMP infusion with routine and biomarker blood tests, abdominal ultrasound, transient elastography and health-related quality of life (HRQL) assessment (full details are provided in the Protocol in the Extended Data).

Transient elastography (Fibroscan®, Echosens, France) is a well-validated non-invasive test to quantify liver fibrosis. It records the velocity of a sound wave passing through the liver and then converts that measurement into a liver stiffness value (expressed in kilopascals (kPa)).[13]

A range of serological biomarker tests are available for assessment of liver fibrosis. We used the Enhanced Liver Fibrosis (ELF™ test (Siemens Healthineers, UK)), a biochemical panel comprising serum markers that are indicators of ECM metabolism (hyaluronic acid, procollagen-III N-terminal pro-peptide (PIIINP), and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1)). The composite ELF score has been validated for detection of liver fibrosis and for prognostication in chronic liver disease.[25,26] By serological assessment of specific ECM fragments it may be possible to separate tissue formation from tissue degradation.[27] We also measured PRO-C3 and C3M (Nordic Bioscience Protein Fingerprint™ technology) which are two markers derived from type-III collagen remodeling, i.e. N-terminal pro-peptide and MMP-9 degraded collagen fragment from the helix region, respectively,[28,29] with utility for staging liver fibrosis and monitoring response to antifibrotic therapy[30,31].

Liver function was assessed by the MELD and the United Kingdom Model for End-Stage Liver Disease (UKELD). These are established clinical scores calculated from objective variables (serum bilirubin, creatinine, International Normalized Ratio (INR) and sodium) that are used to estimate the severity of liver disease, determine prognosis and prioritize patients for transplantation.[32,33]

The Chronic Liver Disease Questionnaire (CLDQ) is a 29-item self-reported disease-specific instrument, measuring HRQL in the following domains: fatigue, activity, emotional function, abdominal symptoms, systemic symptoms, and worry. A composite score is calculated by the patient's response options in each domain using seven-point scales, ranging from the worst (1) to

the best (7) possible function. The CLDQ is reliable, responsive and correlates with the severity of liver disease.[19,34]

Serum cytokines were analysed using a V-PLEX Human Biomarker 54-Plex kit on a MESO Quickplex SQ 120 according to the manufacturers' instructions (Meso Scale Discovery). We selected a set of 6 safety-related cytokines associated with 'cytokine storm' in MAS. These were IL8 (pivotal in the pathogenesis of MAS), IL1α, IL6, TNFα, IFNγ and IL10.

Method of cell production

The monocyte-derived macrophages were manufactured as previously described.[11] Briefly, steady-state leukapheresis was collected from each patient (standard MNC program, 2.5 blood volume). Monocytes were isolated using a CliniMACS Prodigy® cell processor, programme LP14, tubing set TS510 with CliniMACS CD14 Reagent (all Miltenyi). Up to $3.5x10^{10}$ TNC containing $4x10^{9}$ CD14+ cells were processed in a single operation. Mean CD14+ cell purity was 98.3%±0.7% and the mean selection yield was 55.25%±5.4%. A total of $2x10^{9}$ CD14+ cells were cultured in 4x gas-permeable plastic bags (MACS GMP cell differentiation bag 500, Miltenyi Biotec) at $1x10^{6}$ cells per ml in TexMACS GMP (phenol red-free) medium supplemented with 100 ng/mL M-CSF (GMP-grade, R&D systems). Medium was replenished by removing 50% spent medium and replacing with 50% fresh medium supplemented with 200ng/mL M-CSF after 48 and 96 hours of culture. After 7 days, macrophages were harvested, counted and formulated into saline for injection supplemented with 0.5% human serum albumin (Alburex, CSL Behring UK). Macrophages were characterized as viable, CD45+, CD14+, 25F9+ cells as previously described.[11] $CD14^{+}$ monocytes were successfully isolated from all participants. A macrophage product was successfully manufactured and administered for all participants.

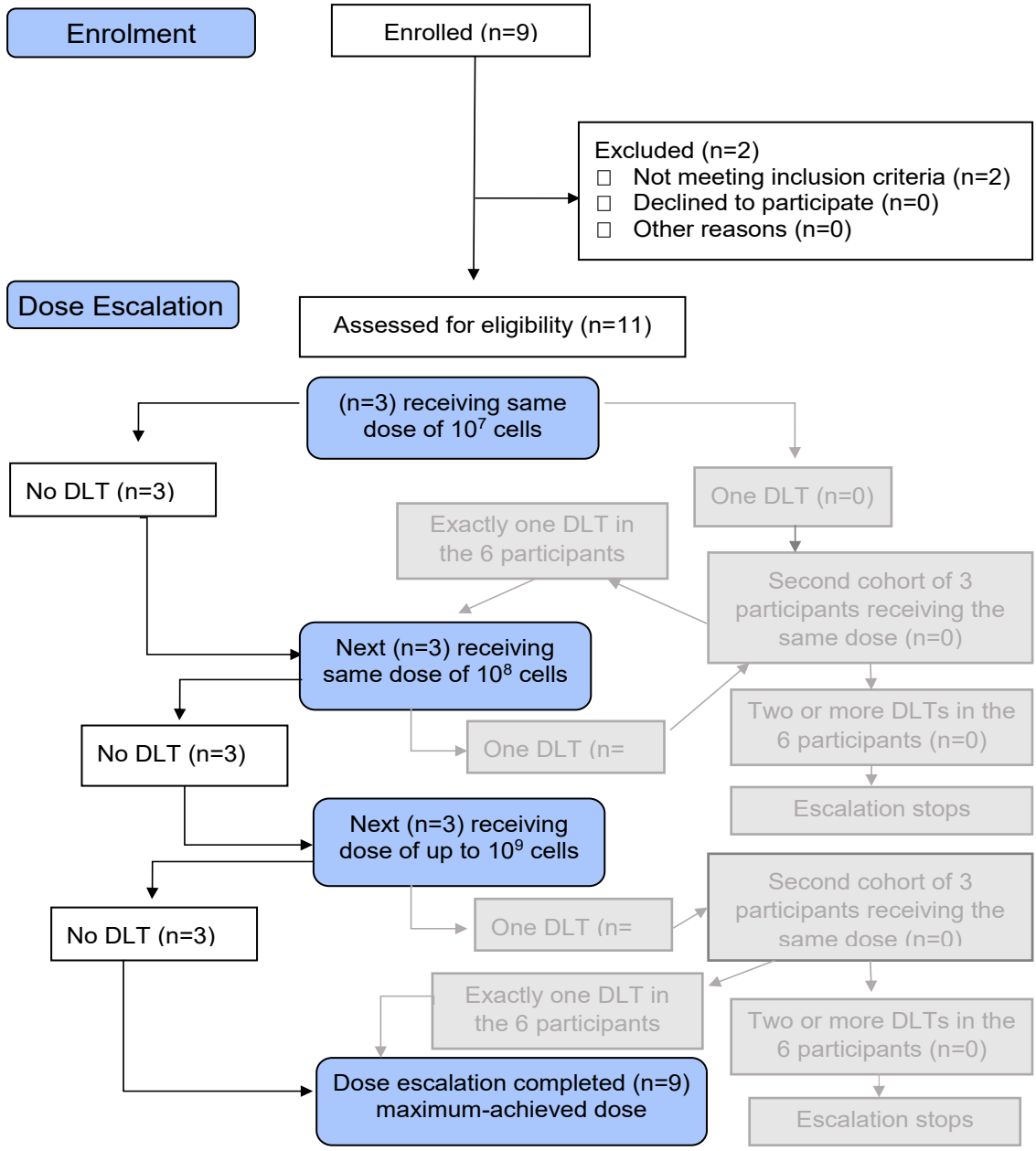
Statistics

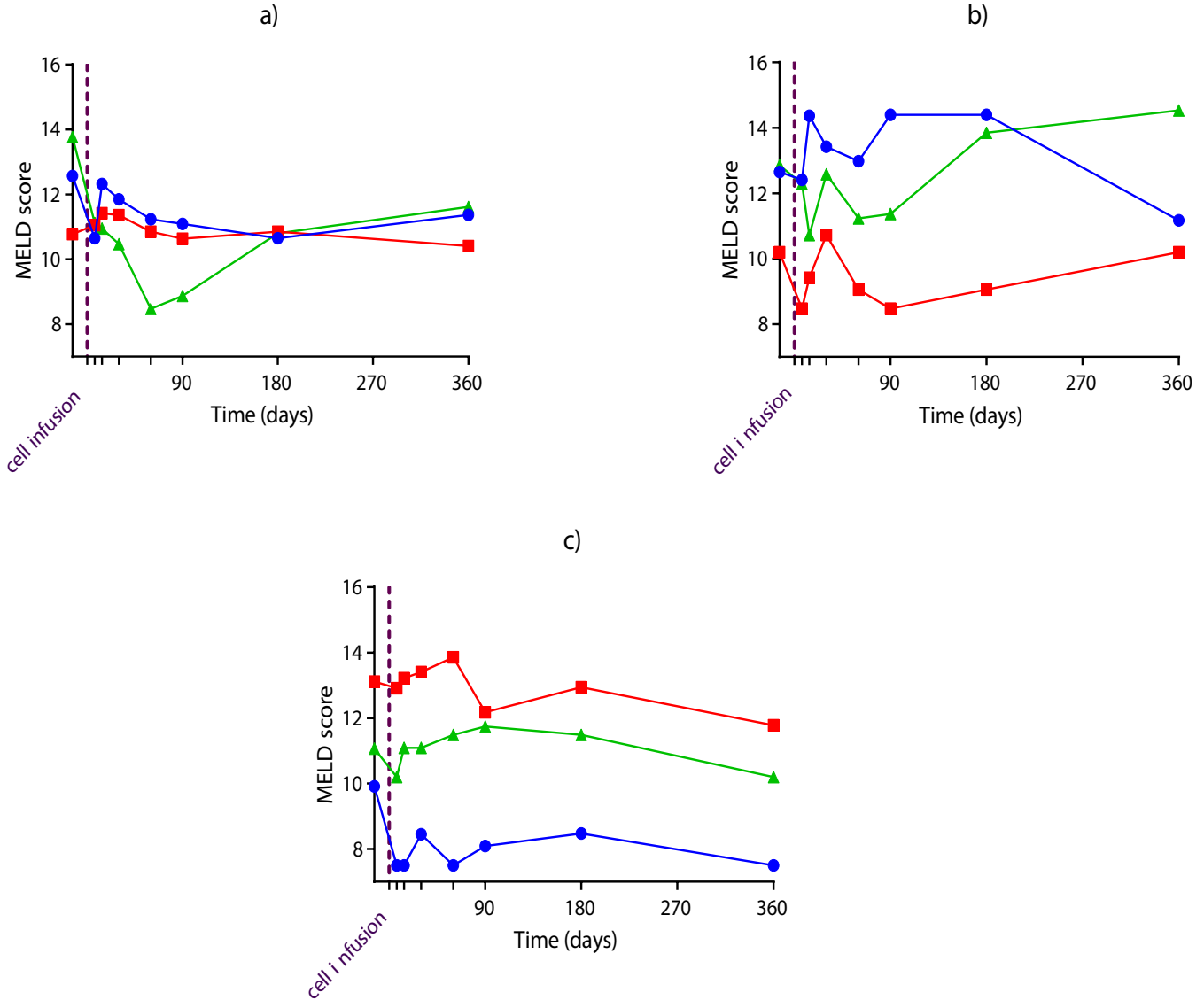
A descriptive analysis of the primary outcome of safety and tolerability is presented. Secondary outcomes are presented graphically by dose and as changes from baseline. Unless stated, numerical data is expressed as mean±standard deviation (SD). A safety report was produced to review the day 14 results of the first participant, thereafter DMC reports were produced following

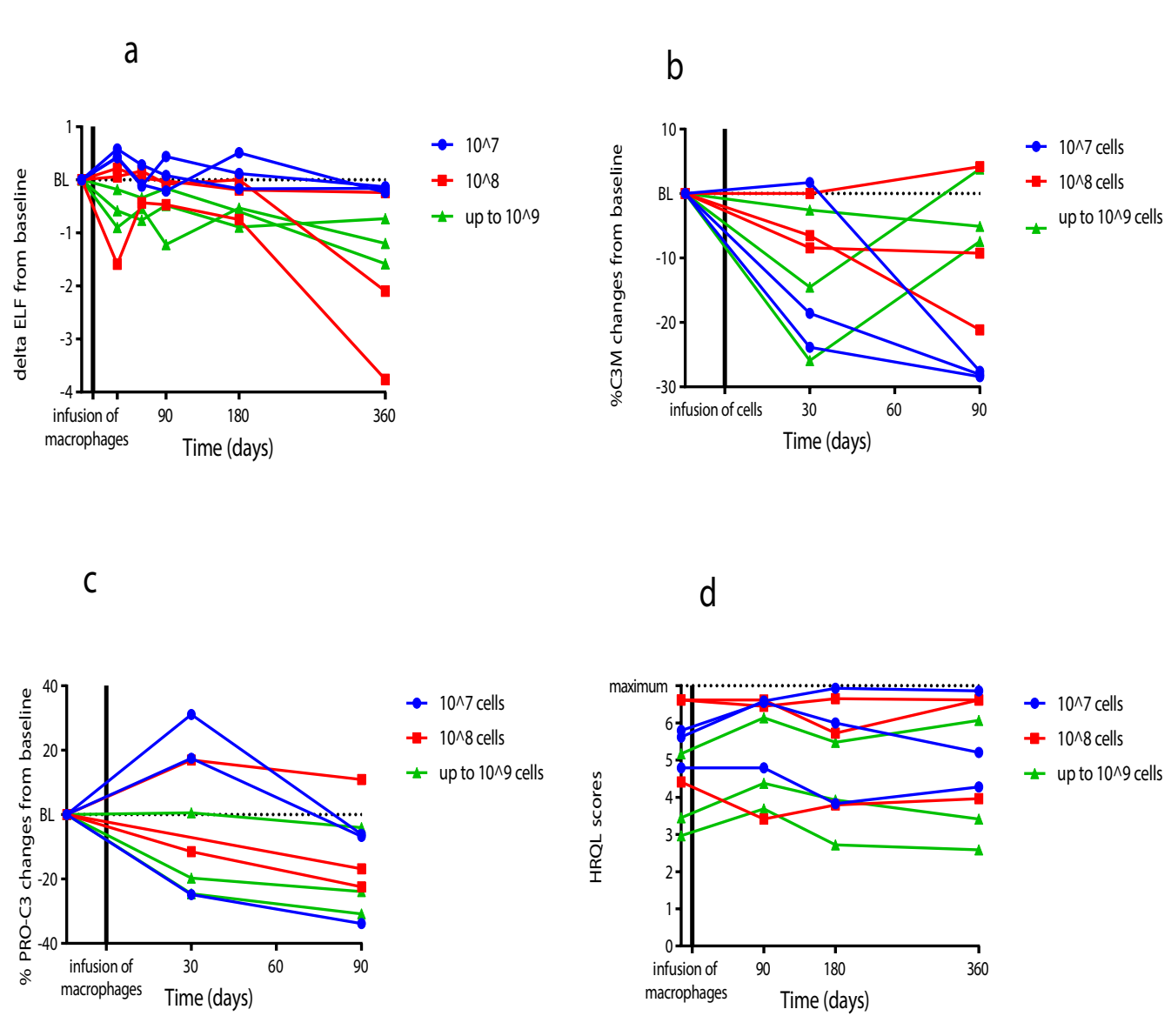
the day 14 safety blood samples of each escalation group of 3 participants at each dose level or as required by serious adverse events. Any additional analysis was performed at the end of the trial once the electronic database was locked following quality checks (QC). There was 100% QC of the data collected, with no missing data other than a single collagen biomarker sample at day 60 post-infusion. We report all adverse events by dose.

Data availability

Data in the published article (and its Supplementary Information files) has been presented where possible in aggregated form. Any data presented to illustrate individual patient performance has been de-identified and only includes analysis of performance within the trial (such as MELD score). The datasets generated during and/or analysed during the current study are available from the corresponding author (SJF) upon reasonable request, although restrictions may apply due to patient privacy and General Data Protection Regulation.


Enrolment
Enrolled (n=9)
Excluded (n=2)
Not meeting inclusion criteria (n=2)
Declined to participate (n=0)
Other reasons (n=0)
Dose Escalation
Assessed for eligibility (n=11)
(n=3) receiving same dose of 10^7 cells
No DLT (n=3)
One DLT (n=0)
Exactly one DLT in the 6 participants
Second cohort of 3 participants receiving the same dose (n=0)
Next (n=3) receiving same dose of 10^8 cells
Two or more DLTs in the 6 participants (n=0)
No DLT (n=3)
One DLT (n=
Escalation stops
Next (n=3) receiving dose of up to 10^9 cells
Second cohort of 3 participants receiving the same dose (n=0)
No DLT (n=3)
One DLT (n=
Exactly one DLT in the 6 participants
Two or more DLTs in the 6 participants (n=0)
Dose escalation completed (n=9) maximum-achieved dose
Escalation stops


a)
16
14
12
10
8
MELD score
90
180
270
360
Time (days)
cell infusion
b)
16
14
12
10
8
MELD score
90
180
270
360
Time (days)
cell i nfusion
c)
16
14
12
10
8
MELD score
90
180
270
360
Time (days)
cell i nfusion


a
delta ELF from baseline
1
BL
-1
-2
-3
-4
infusion of macrophages
90
180
360
Time (days)
10^7
10^8
up to 10^9
b
%C3M changes from baseline
10
BL
-10
-20
-30
infusion of cells
30
60
90
Time (days)
10^7 cells
10^8 cells
up to 10^9 cells
c
% PRO-C3 changes from baseline
40
20
BL
-20
-40
infusion of macrophages
30
60
90
Time (days)
10^7 cells
10^8 cells
up to 10^9 cells
d
HRQL scores
maximum
6
5
4
3
2
1
0
infusion of macrophages
90
180
360
Time (days)
10^7 cells
10^8 cells
up to 10^9 cells