Junta de Andalucía
Consejería de Salud y Familias
Fundación Progreso y Salud

red andaluza
de diseño y traslación de
terapias avanzadas

SUMARY OF THE CMMO/RH/2009 CLINICAL TRIAL RESULTS

A phase II, multicenter, prospective, open-label, randomized, controlled clinical trial to evaluate the safety and signs of efficacy of the use of a single intraportal infusion of autologous bone marrow mononuclear cells as stimulator of hepatic regeneration prior to the realization of extended hepatic resections.

Sponsor: Fundación Progreso y Salud- Red Andaluza de Diseño y Traslación de Terapias Avanzadas

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Abbreviations

AE	Adverse Event
BM-MNC	bone marrow mononuclear cells
CT scan	computerised tomography scanner
DBP	diastolic blood pressure
EFG	epidermal growth factor
HGF	hepatocyte growth factor
IMPD	Investigational Medicinal Product
MedDRA	Medical Dictionary for Regulatory Activities
RHV	Residual Hepatic Volume
SAE	Serious Adverse Events
SBP	systolic blood pressure
SC	Stem Cell
SOL	Space occupying lesions
TGF-a	growth factor alpha
THV	Total Hepatic Volume
USAEs	Unexpected Serious Adverse Events

A. CLINICAL TRIAL INFORMATION

1. Clinical trial identification

Tittle: A phase II, multicenter, prospective, open-label, randomized, controlled clinical trial to evaluate the safety and signs of efficacy of the use of a single intraportal infusion of autologous bone marrow mononuclear cells as stimulator of hepatic regeneration prior to the realization of extended hepatic resections.

2. Identifiers

Protocol code: CMMo-RH-2009

EudraCT: 2009-017793-20

3. Sponsor Data

Andalusian Network for the Design and Translation of the Advanced Therapies (former Andalusian Initiative for Advanced Therapies) through Andalusian Progress and Health Foundation.

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4. Paediatric Data

The clinical trial didn't allow the inclusion of paediatric patients.

5. Analyses Phase of Results

Study start date:

Date of inclusion of the first patient to the study: 03/03/2011.

Completion date of the study:

Date of last visit of the last patient in the trial: 20/05/2015.

6. Clinical Trial general information

6.1Experimental drug

Autologous bone marrow mononuclear cells.

The cell therapy, considered as medicinal product, and subject of this clinical study, consists of a suspension of autologous bone marrow mononuclear cells.

The BM aspirate was collected in aseptic conditions in a transference bag containing anticoagulant ACD-A solution in a proportion of 1:5 of the BM volume. Removal of plasma, red blood cells and granulocytes was performed, keeping exclusively the BM-MNC. The procedure was carried out by a density gradient centrifugation on Ficoll-Hypaque density 1077, using a SEPAX automatic cell processor. The BM-MNC suspension was washed twice with human albumin at 4% in the same machine in order to eliminate the Ficoll. After the two washes, cells were subjected to a last centrifugation and re-suspended in 10 to 30 ml of a 0.9% NaCl sterile solution. The IMP was administered via intraportal vein, as a single infusion following embolization of affected segments.

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6.4Pathology study

Space occupying hepatic lesions who required an extended hepatic resection (over five segments), with a residual hepatic volume insufficient to ensure the hepatic function and/or the necessary safety margins after resection.

6.5General study desing and description of the research

A phase II, prospective, multicentre, open-label, randomised, controlled clinical trial to evaluate the safety and signs of efficacy of the use of a single intraportal infusion of autologous bone marrow mononuclear cells as stimulator of hepatic regeneration prior to the realization of extended hepatic resections.

It was designed with two experimental groups of treatment:

Study group: patients with hepatic SOL that require an extended hepatic resection to whom, prior to surgery, a portal embolization of the affected segments, as well as an intraportal application of BM-MNC.

Control group: patients with hepatic SOL that require an extended hepatic resection to whom, prior to surgery, a portal embolization of the affected segments.

The distribution of cases/controls was 1/1.

6.5.1. Timeline

According to with the trial design, patients had to attend to, at least, 10 visits throughout the trial. The first visit (V1) was for the recruitment. This visit was scheduled no more than one month before embolization. Patients had to sign the consent form and, any procedure needed to assess the recruitment criteria according to the protocol was implemented including a baseline Helical CT scan. In the second visit (V2), BM extraction, embolization of the portal branches affected by the lesion and infusion of the BM-MNC into the portal vein was carried out. Visit 3 was scheduled at 24

hours after the infusion and embolization. Visits 4 and 5 were scheduled 2 and 4 weeks after embolization and the effect was measured by Helical CT scan; a 6th visit was optional only for those patients who required a second volumetric evaluation prior to the hepatic resection. Visit 7th was the preoperative evaluation (15 days after visit 5 or 6) to assess the patient condition prior to surgery. Subsequently, visit 8 included patient admission, surgery, and postoperative monitoring. Finally, visits 9, 10 and 11 were scheduled at 4 and 12 weeks, and 12 months after surgery, respectively, with a volumetric assessment by Helical-CT carried out in visit 9.

6.6 Scientific background and justification

Hepatic regeneration is a fundamental response of the liver to tissue damage. The complex interaction of factors which determine this response includes a stimulus (experimentally, a hepatectomy), genes expression, synthesis of various growth factors and the interaction of others factors which modulate the response. The study of the phenomenon has allowed to find a number of clues that help to understand the organogenesis and how the signs that determine the observed responses are produced[1,2].

In 1931, Higgins and Anderson observed, in rats subjected to a partial hepatectomy of 2/3 of the liver, leaving the remaining lobes intact, that these grew quickly until restoring the original hepatic mass in 5 to 7 days[3]. Other studies in dogs, monkeys[4], and humans have demostrated that the regenerative response is proportional to the amount of removed liver removed[5]. Even small resections of less than 10% of the hepatic mass, result in a response, which leads to a recovery of the original size. Studies prove that the hepatic mass is regulated by signals that can have either positive or negative effects on that mass.[6, 7]

Unlike other tissues, the liver does not depend on a germinal cell group (stem cells) for regeneration, relying on the proliferation of all the remaining mature cells: hepatocytes (main functional cells), biliary epithelial cells (that cover the canaliculi), fenestrated endothelial cells (that cover the hepatic sinusoids and allow a direct exchange between blood and hepatocytes), Kupffer cells (macrophages in the sinusoids) and the Ito cells (star-shaped cells whose long processes cover the hepatocytes, store A vitamin, synthesize different proteins of the connective tissue and secrete different growth factors). All spread to restore the lost tissue, although the speed of response of each type of cell is different.1,2 Studies with hepatic cell cultures have shown that under the stimulus of the hepatocyte growth factor, transforming growth factor alpha and

epidermal growth factor, the hepatocytes undergo first a process of dedifferentiation and then of re-differentiation to make either mature hepatocytes or structures similar to biliary ducts.[8]

The trigger for the regenerative response, the regulation of the phases that participate in such response and the termination of the process are not completely understood, although several studies have allowed to establish a series of facts.

The first one is that the mitogenic signal is induced by factors, which appear rapidly in the blood and are active on remaining hepatocytes, stimulating their proliferation. Some growth factors such as HGF, EGF and TGF-a, are directly involved in the mitogenic response while a number of factors act indirectly; however, their presence is also indispensable to generate appropriate mitogenic stimuli. Hepatic tissue transplanted to (ectopic) extrahepatic sites also responds and increases the synthesis of DNA after the hepatectomy.[2,8]

Currently, an approach for patients with space occupying hepatic lesions requiring an extended hepatectomy, is to carry out a preoperative embolization of the segments where the lesion is, to facilitate the regeneration of the remaining segments in order to allow a safer surgery. Regarding hepatic resections, the concept of "pre-operative expansion" of the left lateral hepatic remaining segments (II and III) using a selective portal embolization of the contralateral liver segments (I and IV to VIII) prior to a right tri-segmentectomy, is considered a safe and efficient method to provide the necessary stimulus for hepatic regeneration. However, the response to contralateral portal embolization is slow, often requiring more than 150 days to achieve an optimal growth of the left hepatic segments and thus results in an excessive tumour growth preventing to obtain a functional liver remnant and restricting tumour-free resection margins.[9–13]

In this context, the prospect of cellular regeneration therapy is of great interest. In an attempt to improve the results of portal embolization techniques, and accelerate liver proliferation, the use of extrahepatic stem cells, such as hematopoietic progenitor cells, has been studied.[14,15] Recently, treatment by implanting progenitor adult cells derived from bone marrow on portal branches of the remaining segments has been investigated with promising results.[16–18]

Thus, the hypothesis of the trial is that BM-MNC infused in the portal territory of the remaining hepatic segments (II y III), at the same time as a contralateral portal embolization, will enhance hepatic regenerative capacity, shorten the hepatic regeneration time and increase the residual

volume. As a result, it would make it safer to carry out an extended hepatectomy, with more guarantees of maintaining a correct residual liver function and adequate surgical margins.

6.7Clinical trial objectives.

Main objective.

To assess the feasibility and safety of autologous bone marrow mononuclear cells as enhancer of hepatic regeneration.

Secondary objectives.

1.- To assess the complications derived from the regenerative therapy and/or the study procedures

2.- To compare the increase of the volume obtained after the application of the hepatic regeneration procedures prior to surgery.

3.- To determine the percentage of resections that has allowed the hepatic regeneration.

4.- To assess hepatic functional grade after surgery.

6.8 Withdrawal Criteria

Based on the protocol guidelines, patients were required to discontinue the clinical trial if at least one of the following situations occurred:

1. In case that the volume of the extracted BM wasn't at least of 200-250 cc to get a sufficient volume of cellular suspension. The researcher would decide on the administration, although the patient would be excluded from the analysis of safety/feasibility.

2. Presence of serious adverse event that would compromise the patient' s life from the moment of inclusion in the study (by singing the informed consent) to the infusion of the BM-MNCs.

3. Patient clinical conditions that prevent him/her from continuing.

4. Additionally, subject could be excluded from the study by the following reasons:

- When patient does not cooperate or does not meet the study requirements.
- When the researcher may consider that patient' s health could be at risk due to adverse reactions, concomitant diseases or any other circumstances that may occur during the study.
- Adverse event (s)
- Abnormal laboratory value(s) with clinical meaning.
- Abnormal results from the tests procedures
- Protocol violation
- Withdrawal of consent by the patient
- Patient lost to follow-up

5. Presence of active infection or wet gangrene at the BM-MNCs infusion day

According to good clinical practice, an alternative treatment should be recommended to all patients who had prematurely left the study. Additionally, if the withdrawal was due to a significant adverse event, patients should be monitored by the researcher until proper completion, that is, until the adverse event disappears or is resolved as permanent.

6.9 Experimental drug

The investigational medicinal product (IMP) is an advanced therapy medicinal product that consists of a suspension of autologous bone marrow mononuclear cells.

The BM aspirate was collected in aseptic conditions in a transference bag containing anticoagulant ACD-A solution in a proportion of 1:5 of the BM volume. Removal of plasma, red blood cells and granulocytes was performed, keeping exclusively the BM-MNC. The procedure was carried out by a density gradient centrifugation on Ficoll-Hypaque density 1077, using a SEPAX automatic cell processor. The BM-MNC suspension was washed twice with human albumin at 4% in the same machine in order to eliminate the Ficoll. After the two washes, cells were subjected to a last centrifugation and re-suspended in 10 to 30 ml of a 0.9% NaCl sterile solution.

The BM fractions were separated to obtain the MNC fraction that was transferred to the radiology suite for immediate administration of the IMP to the patient. On average 45.8 ± 6.57ml (35-50) were obtained containing 1460 x 10^6 ± 607 x 10^6 MNC. Viability was 96.8% (94-98%). In the first 2 cases (D01 and D02) the cells were diluted before the infusion in sterile saline solution 1:3. Individual values are shown in Table 1.

Stem cells application

After the embolization of the portal branches where the lesion is located, we proceeded to the*selective application of the stem cells into the portal branches of the remaining liver segments in the study group. The application was made using a 5F catheter Cobra (Terumo) inserted in the branches of the remaining hepatic segments under fluoroscopy control. The IMP was slowly infused for 15 minutes.

Tabla 1. Individual results of obtained IMP (BM-MNC)

Patient	*Total MNC (x10^6)*	*Viability (%)*	*Viable MNC (x10^6)*	*Volume (ml)*
EC08D01	***975***	***97***	***945.75***	***150***
EC08D02	***990***	***98***	***970.2***	***150***
ECO8D08	***1100***	***94***	***1034***	***50***
EC08D11	***2006.4***	***97***	***1946.208***	***44***
ECD08D13	***2229.5***	***98***	***2184.91***	***35***

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6.10 Statistical methods

Data analysis was carried out by R statistical software (R Core Team 2019), and the RStudio interface (version 1.2.1335). R packages were used for descriptive analysis of each variable, contingency analysis, non-parametric tests for comparison between groups, and mixed models for the effect of time and group in efficacy variables. Volumetric and biopsy data were analyzed and plotted using Prism software (GraphPad prism V8).

6.10.2. Descriptive analysis

Descriptive analysis was carried out for all collected demographic variables, as well as for baseline clinical data, in the intention to treat population (all randomized patients). Qualitative variables are expressed in absolute frequencies and percentages whereas quantitative variables are presented by mean and standard deviation, maximum, minimum, and number of observations. The method for missing values treatment was listwise deletion, unless indicated otherwise in the corresponding section.

7. Población de los sujetos de ensayo

The population consisted of patients with space occupying hepatic lesions who required an extended hepatic resection, with the residual hepatic volume insufficient to ensure either the hepatic functionality and the safety margins necessary after resection, of both sexes and with an age equal or higher than 18 complying the selection criteria detailed below.

B. DISPOSITION OF THE SUBJECTS OF THE TRIAL

1. RECRUITMENT

Patients were recruited within the General Surgery and Digestive System Services of Hospital Virgen del Rocío and after signing the informed consent, all tests necessary to confirm their compliance with the selection criteria were performed.

14 patients were included (after signing the consent) being one a selection fault and, consequently, 13 measurable patients according to the definition of population by intention of being treated (Figure 1).

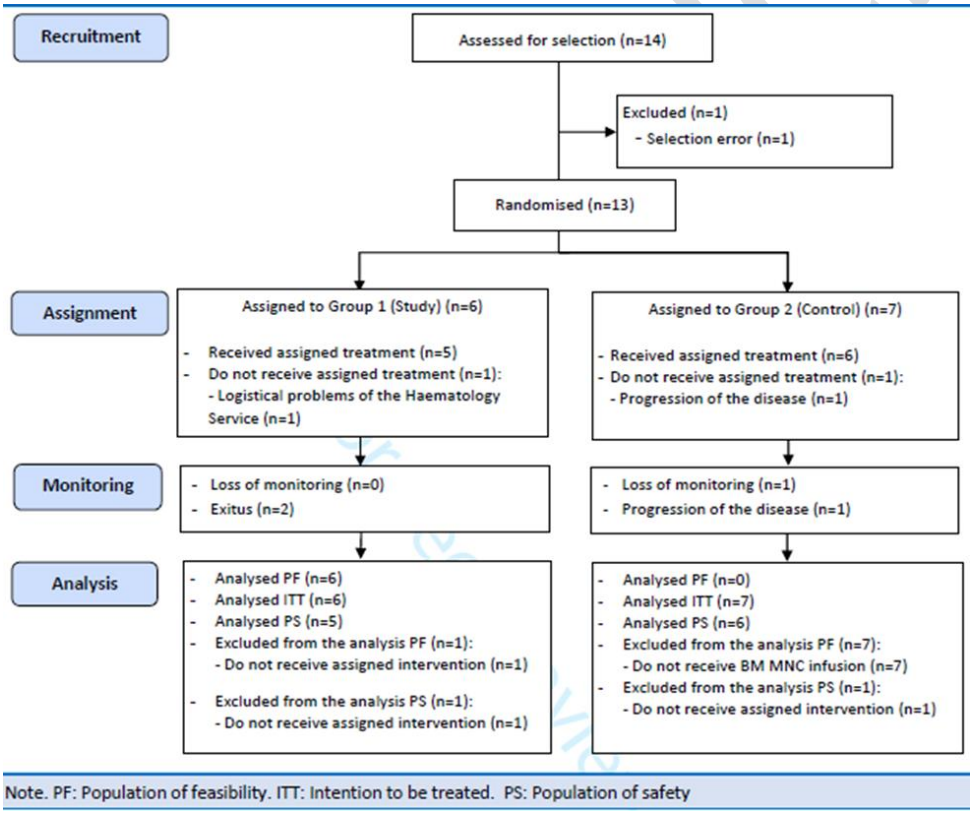

Figura 1. Flowchart

As regards the 13 patients who were randomised, two patients didn’t receive treatment, one from the study group due to “logistical problems of the Haematology Service” and the other

patient from the control group due to "progression of the disease in pre-embolization evaluation, excluding an indication of embolization or surgery"

1.1 Inclusion criteria

1. Both genders patients, age ≥ 18 years' old
2. Normal analytic parameters, defined by:

Leucocytes ≥ 3000

Neutrophils ≥ 1500

Platelets ≥ 100000

AST/ALT ≤ 1.5 institution standard range

Creatinine ≤ 1.5 mg/dl

3. Patients with space occupying hepatic lesions (SOL) who required an extended hepatic resection. Selection was based on a preoperative evaluation of residual hepatic volume after hepatectomy of less than 30% (40% in diseased liver). The selection of patients comprised 4 types of liver lesions:

- Subsidiary metastatic disease of right hepatectomy enlarged to segment IV or with suspicion of diseased liver (neoadjuvant chemotherapy) (in cases of doubt of liver function, the "indocyanine green" test may be used)
- Bilobar liver metastases with multiple right lobe nodules and more than 3 nodules greater than 30 mm in left hepatic lobe: LHI tumorectomies + right portal branch ligation (or postoperative percutaneous embolization) should be performed with a view to performing right hepatectomy at 4-6 weeks ("two stage" surgery)
- Hepatocarcinoma on cirrhotic liver subsidiary of right hepatectomy
- Benign Hepatic Lesions (Hemangiomas, Hydatid Cysts, or Primary Hepatic Tumors / Hepatoblastoma), which, due to their extension, jeopardize the viability of the remaining liver tissue.

4. Patients who give, by writing, their informed consent to participate in the study and who provide sufficient guarantees of adherence to the protocol in the opinion of the researcher responsible for patient assistance.
5. Women of childbearing age had to obtain negative results by a serum pregnancy test took at the moment of inclusion in the study, according to each hospital usual

procedure, and they had to take the commitment of using a double method of control birth such as an IUD or oral contraceptive plus prophylactic throughout all the study.

1.2 Exclusion Criteria

1. History of neoplasm different to the existing disease or any hematologic disease.
2. Patients with uncontrolled high blood pressure.
3. Severe heart failure (NYHA IV).
4. Patients with malignant ventricular arrhythmias or unstable angina.
5. Diagnosis of deep vein thrombosis in the three previous months.
6. Concomitant therapy that includes hyperbaric oxygen, vasoactive substances, anti-angiogenesis agents or Cox-II suppressors.
7. Body mass index > 40 Kg/m2.
8. Patients with alcoholic liver disease with active alcoholism.
9. Proliferative retinopathy.
10. Concomitant disease that reduces the life expectancy to less than a year.
11. Difficulty to follow up.
12. Heart failure or EF < 30%.
13. Cerebrovascular accident or heart attack in the last 3 months.
14. Pregnant women or of childbearing age without adequate contraceptive method.

1.3 Randomisation

Patients who complied with eligibility criteria and expressed their consent to participate in the trial by signing the informed consent, were randomised either to the placebo group or to the study group. Patients were assigned to the experimental or placebo group by the sponsor using a computer-based random allocation sequence-generating program (Spanish adaptation of the free OxMaR software for minimization and randomization of clinical studies), without any restriction (type of Randomization: 1:1. There were not blocking).

Distribution of cases/controls will be 1/1.

Although the population of the study was going to comprise 65 patients divided into two groups, finally, the population to be studied consisted of 14 patients, of whom one patient was excluded as a selection failure. The number of patients included in this analysis, according to the

definition of population by intention of being treated, was of 13. Seven patients were appointed to the control group and 6 to the study group (Figure 1).

1.4 Masking/Blinding

There are no masking techniques as this is an open-label trial.

C. BASAL CHARACTERISTIQUES

1. General characteristiques

Besides a descriptive statistical analysis of each parameter describing the sample, an assessment between groups was carried out. Groups were homogenous with respect to the variables: age were homogenous in terms of age (mean 59.67±6.68 vs 58.00±14.62, p=0.803), sex (3 male and 3 female vs 4 male and 3 female), weight, height, and BMI.

Concerning biochemical parameters, overall, there were no clinically relevant values in those patients with available data (except for one patient in the control group, EC08D03, who showed clinically relevant values of ALT). Regarding basal measurements of vital signs such as heart rate, systolic blood pressure and diastolic blood pressure, significant differences were found only for heart rate values, where the study group showed a significantly higher average than the control group (84.17±4.12 vs 70.80±2.77, $p < 0.001$). The effect size (calculated with Hedges' g) was g=3.19. Significant differences in variability were found on ultra-sensitive CRP (Table 2).No significant association was observed between randomization groups and the presence of clinically relevant alterations of basal coagulation tests or the baseline hemogram.

Likewise, vascular risk factors including high blood pressure, dyslipidemia, smoking, alcohol consumption, and history of ischemic cardiomyopathy were also assessed and no significant differences were found between groups.

Tabla 2. Baseline Characteristics

Parameters	Group	Mean	SD	Min	Max	Lost Values	p-value
Age (years)	Study	59.67	6.68	49	67	0	0.803
	Control	58.00	14.62	34	75	0	
BMI	Study	24.07	3.74	20.8	31.3	0	0.971
	Control	24.00	2.77	21.1	28.7	0	
Glucose (mg/dl)	Study	111.00	30.32	80	162	0	0.549
	Control	100.71	29.51	81	162	0	
Creatinine(mg/dl)	Study	0.80	0.15	0.65	1.06	0	0.769
	Control	0.83	0.25	0.48	1.29	0	
Total Cholesterol (mg/dl)	Study	231.00	45.42	186	300	1	0.172
	Control	182.17	60.47	102	284	1	
Triglycerides (mg/dl)	Study	100.20	39.86	163	107	1	0.488
	Control	137.17	107.4	45	327	1	
Ultra-sensitive CRP (mg/l)	Study	1.77	1.49	0.50	3.30	2	0.080
	Control	21.90	19.29	2.60	53.90	2	
Total Proteins (g/dl)	Study	7.32	0.63	6.50	8.00	1	0.535
	Control	7.03	0.86	5.70	8.30	0	
Albumin (mg/dl)	Study	4.07	0.42	3.60	4.40	3	0.154
	Control	3.46	0.55	2.50	3.80	2	
Sodium (mEq/l)	Study	140.50	3.56	137	145	0	0.327
	Control	138.83	1.72	137	142	1	
Potassium (mEq/l)	Study	4.79	0.64	4.00	5.83	0	0.094
	Control	4.18	0.48	3.60	4.70	1	
AST (UI/l)	Study	66.66	72.95	17	195	1	0.708
	Control	51.67	55.53	15	160	1	

Parameters	Group	Mean	SD	Min	Max	Lost Values	p-value
ALT (UI/l)	Study	54.00	53.92	11	161	0	0.512
	Control	85.00	99.92	11	282	0	
Alkaline phosphatase (UI/l)	Study	244.00	204.83	71	537	2	0.942
	Control	258.75	331.12	62	751	3	
GGT (UI/l)	Study	203.20	206.69	39	552	1	0.831
	Control	242.17	345.53	19	889	1	
Total Bilirubin (mg/dl)	Study	0.98	0.49	0.38	1.71	0	0.532
	Control	0.77	0.69	0.32	2.24	0	
Heart rate	Study	84.17	4.12	80	92	0	< 0.001
	Control	70.80	2.77	68	75	0	

2. Specific characteristics of the study

At baseline, there were no significant differences between the study and the control groups in the volumetric helical CT variables, RHV and THV.

Tabla 3 Baseline volumetric helical-CT

Group	Patient	Total THV (cc)	Residual RHV (cc)	Ratio RHV/THV (%)
Control	EC08D03	2176	555	25.51
	EC08D05	2356	1592	67.57
	EC08D06	1131	381	33.69
	EC08D07	1619	584	36.07
	EC08D10	1404	515	36.68
	EC08D12	1293	291	22.51
Mean (± SD)		1663.17 (±496.46)	653.00 (±473.32)	37 (±16)
Study	EC08D01	1229	566	46.05
	EC08D02	948	769	81.12
	EC08D08	1145	445	38.86

	EC08D11	1846	361	19.56
	EC08D13	2201	807	36.67
Mean (± SD)		1473.80 (±527.22)	589.60 (±195.69)	44 (±23)

No differences were found in the number of affected hepatic segments prior to surgery (Table 4).

Tabla 4. Involvement of hepatic segments

Randomisation Group	**Patient**	**Segments**							
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**
Study	EC08D01								
	EC08D02								
	EC08D04								
	EC08D08								
	EC08D11								
	EC08D13								
Control	EC08D03								
	EC08D05								
	EC08D06								
	EC08D07								
	EC08D09*								
	EC08D10								
	EC08D12								

Note: Healthy segments are highlighted in green and affected ones in red. *excluded due to disease progression

D. ASSESSMENT CRITERIA

1. Definitions

Feasibility was evaluated considering the percentage of patients assigned to the study group who completed the procedure, i.e. an autologous infusion of BM-MNC at the time of portal embolization in the absence of serious adverse events, in visit 2.

Safety was evaluated taking into account all adverse events, their severity, relation with the IMP, and unexpected serious adverse events in each group. The ratio to complete the surgery and the number and cause of exitus was also recorded. Safety was evaluated in all patients who received their assigned treatment and the results in both groups were compared.

Regarding efficacy, several criteria were evaluated to assess the regenerative response in both groups. These criteria included volumetric helical CT measurements at two time points, percentage of patients that completed surgery in each group, tumor-free resection margins (in mm), structural histological evaluation of the regenerated hepatic tissue and grade of fibrosis.

2. Feasibility

Bone marrow collection was performed in an operating room by repeated aspiration of the posterior iliac crest under local anesthesia and sedation. The BM fractions were separated to obtain the MNC fraction that was transferred to the radiology suite for immediate administration of the IMP to the patient. On average 45.8 ± 6.57ml (35-50) were obtained containing 1460 x10^6 ± 607x 10^6 MNC and viability was 96.8% (94-98%). In one patient it was not possible to schedule the BM collection. The BM fractions were separated to obtain the MNC fraction that was transferred to the radiology suite for immediate administration of the IMP to the patient.

After embolization of the portal branches where the lesion was located, we proceeded to the selective application of the BM-MNC solution in the portal branches of the remaining liver segments in the patients assigned to the study group. Following intrahepatic application of the cells, the portal catheter was withdrawn and the patient was continuously monitored during 24

hours. The procedure was completed in 83.33% of patients assigned to the study group (5/6). Only one patient failed to complete the procedure due to a logistic issue, as mentioned above, that was unrelated to the patient´s clinical condition at the time of the procedure.

Adverse events (AE) and serious adverse events (SAE) related to the treatment with BM-MNC were assessed in the first 24 hours and during the follow-up period at 2, 4 and 6 weeks after its administration. There were no SAE related to the procedure of either BM extraction or the administration of BM-MNC. Two minor AE, pain in the hypochondrium and pain in the right hemithorax, appeared after the administration of the IMP but were related to the procedure and not to the product itself. In Visit 3, day 1 after the administration of BM-MNC, 4 AE were registered, representing 4.87% of total AE occurred during the clinical trial. In Visit 4, second week after administration of BM-MNC, a total of 3 AE were registered, being a 3.66% of the total. Regarding Visit 5, 4 weeks after the administration, only one AE was registered, representing 1.21% of the total. Finally, at week 6 (including data from Visit 6 and Visit 7), 2 AE were registered representing 2.44% of total adverse events. None of the reported AE was considered related to the IMP.

Thus, the analysis of feasibility showed that an autologous infusion of BM-MNC at the time of portal embolization is feasible, given the high percentage of patients that completed the whole procedure, in the absence of serious AEs.

3. Efficacy

3.1 Volumetric Helical CT variables

Volumetric Helical-CT was acquired using the Syngo.via (Siemens) software following standard procedures:10 mm thick sections with 10 mm reconstruction were collected in axial view at visit 1 (baseline), visit 5, (optional at visit 7) and visit 9-10. For each axial section the volumes were calculated as Volume (cubic centimeters, cc) = Area (cm2) x Reconstruction Index (cm). Volumes were calculated by adding up the area of each section for Total Hepatic Volume (THV) and Residual Hepatic Volume (RHV) and the ratios RHV/THV. To compare the effects of intervention the ratio of RHV to THV at baseline (THV0) was used. The radiologist (HP-V) was blind investigator.

At baseline, there were no significant differences between the study and the control groups in the volumetric helical CT variables, RHV and THV (Table 3). Individual values foreach patient at one month after the embolization (V5) and after the hepatectomy are shown in Table 5. To assess the

effect of both interventions, the ratio of residual volume to total baseline volume was used (RHV/THV0). Analysis of the volumetric data acquired at baseline, post-embolization and post-hepatectomy showed a significant effect of time (mixed models effects, F2, 14= 13.99 p = 0.0005), but not of treatment group (p = 0.6), in the extent of hepatic regeneration (Figure 2). There were no significant time by group interactions (p = 0.72). Of note, there were very significant within group differences, as expected given the heterogeneity of the lesions, and very effective matching, suggesting that the extent of regeneration was dependent on the initial residual volume. There were no differences between the study and control groups in the time to achieve hepatic regeneration.

Tabla 5. Volumetric helical-CT at post-embolization and post-hepatectomy

Group	**Patient**	**Post-embolization**		**Post-hepatectomy**
		THV (cc)	**RHV (cc)**	**THV = RHV (cc)**
Control	EC08D03	1525	575	976
	EC08D05	2143	1591	1664
	EC08D06	1186	493	913
	EC08D07	1477	525	1242
	EC08D10	1463	648	906
	EC08D12	1164	354	-
Mean (± SD)		1493 (±354.17)	697.67 (±448.41)	1140.2 (±323.33)
Study	EC08D01	1228	591	-
	EC08D02	1077	920	1099
	EC08D08	1157	688	-
	EC08D11	1368	393	1152
	EC08D13	3164	1032	886
Mean (± SD)		1207.5 (±123.50)	724.8 (±255.84)	1045.67(±140.79)

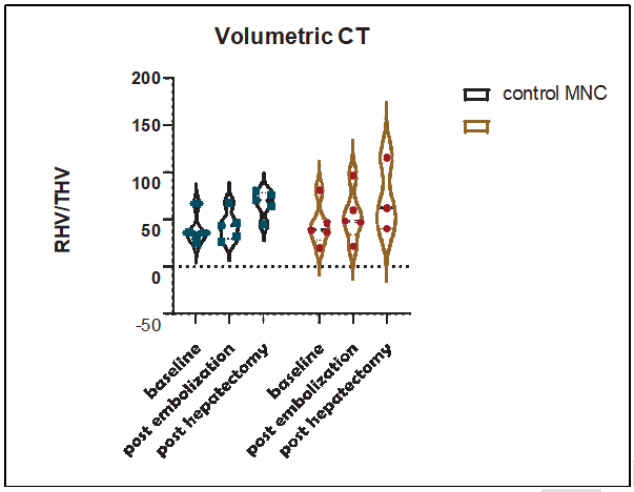

Figura 2. Analysis of the volumetric data acquired at baseline, post-embolization and post-hepatectomy

3.2 Histological analysis of the hepatic biopsy

The tumor-free resection margins were measured in all the samples taken at the time of the hepatectomy (N=5/5). The results of the histological analysis showed no differences between the groups. Only one patient in the study group showed tumor infiltration at the resection margins. The grade of fibrosis was also evaluated following the Metavir classification27. This classification of fibrosis is divided into 5 categories, F0 to F4. The majority of patients showed an F0 level, or absence of fibrosis; however, two cases reached grade F4 or cirrhosis. In addition, 2 patients in the control group had mild or moderate steatosis but none exceeded the 30% cut-off point.

E. Acontecimientos Adversos

1. Percentage of patients in whom surgery could be performed

According to the analysis plan, the percentage of patients who reached V8 was studied. Five patients in the study group (83.33%) and 5 patients in the control group (71.43%) reached visit 8 and received surgery as scheduled with no differences between groups (=0.258, p = 0.611, Fisher' s exact test). In the study group one patient did not receive the assigned intervention and left the study. In the control group one patient did not receive the assigned intervention and one

patient showed disease progression in the post-embolization control and was withdrawn from the study.

2. Adverse events. Encoding

In total, 82 AE occurred, 39 in the study group (8 ± 8.5 events per patient) and 43 in the control group (7 ± 4 events per patient), odds ratio of 0.907. The extracted information from the trial database was coded according to the terminology from MedDRA version 16.1 dictionary with levels SOC, HLGT, HLT, PT, LLT, in order to homogenize the terms and facilitate the analysis (Table 6). 75 out of the 82 adverse events that occurred throughout the trial were non-serious, 39 in the control group versus 34 in the study group. All patients included in the safety population suffered at least one AE except for patient EC08D02 (study group). Most frequent AE were anaemia (6.10%), diarrhea (4.88%), asthenia (3.66%), neutropenia (3.66%), and vomits (3,66%).

Tabla 6. Adverse Event list per patient according to MedDra terms

Group	Patient code	Adverse Event	TP Term	TP Code	SOC Term	SOC
Control	EC08D03	Chest pain	Chest pain	10008479	Heart disorders	10007541
		Severe anemia	Anemia	10002034	Blood and	10005329
		Hemoperitoneum	Peritoneal	10034666	Gastrointestinal	10017947
		Asthenia	Asthenia	10003549	General	10018065
		Toxicity in hand	Skin toxicity	10059516	Skin and	10040785
		Anorexia	Decreased appetite	10061428	Nutrition and	10027433
		Neutropenia	Neutropenia	10029354	Blood and	10005329
	EC08D05	Thyroid cysts	Cysts of thyroids	10043706	Endocrine	10014698
		Dilated Ischemic myocardiopathy	Congestive	10056370	Heart disorders	10007541
	EC08D06	Diarrhea	Diarrhea	10012735	Gastrointestinal	10017947
		Mucositis	Inflammation of	10028116	General	10018065
		Alopecia	Alopecia	10001760	Skin and	10040785
		Insomnia	Insomnia	10022437	Psychiatric	10037175
		Skin hyperpigmentation	Skin	10040865	Skin and	10040785
		Facial contusion by fall	Contusion	10050584	Traumatic	10022117
		Hematuria	Hematuria	10018867	Kidney and	10038359
		Asthenia	Asthenia	10003549	General	10018065
		Nauseas	Nauseas	10028813	Gastrointestinal	10017947
		Neutropenia	Neutropenia	10029354	Blood and	10005329
	EC08D07	Agitation y disorientation	Agitation	10001497	Psychiatric	10037175
			Disorientation	10013395	Psychiatric	10037175
		High Blood Pressure	High Pressure	10020772	Vascular	10047065
		GPT increase	High alanine	10001551	Complementary	10022891
		Ascites	Ascites	10003445	Gastrointestinal	10017947
		Hypoproteinemia	Hypoproteinemia	10021083	Nutrition and	10027433
		Hypophosphatemia	Decreased	10049471	Complementary	10022891
		Febrile Syndrome	Pyrexia	10037660	General	10018065
		Dysuria	Dysuria	10013990	Kidney and	10038359
		Oedemas on lower limbs	Peripheral oedema	10030124	General	10018065
		Bradycardia (Iatrogenesis by Diltiazem)	Bradycardia	10006093	Heart disorders	10007541
		Anemia	Anemia	10002034	Blood and	10005329
		Thrombopenia	Thrombocytopenia	10043554	Blood and	10005329
	EC08D10	Fever (after embolization)	Pyrexia	10037660	General	10018065
		Intra-abdominal biliary collection	Allocated	10066768	Gastrointestinal	10017947
		Diarrhea	Diarrhea	10012735	Gastrointestinal	10017947
		edema in right leg	Peripheral edema	10030124	General	10018065
		Right pleural effusion	Pleural effusion	10035598	Respiratory,	10038738

		Persistence	Collection of intra-	10078659	Gastrointestinal	10017947
		Anemia	Anemia	10002034	Blood and	10005329
		Abdominal pain	Abdominal pain	10000081	Gastrointestinal	10017947
		Diarrhea	Diarroea	10012735	Gastrointestinal	10017947
	EC08D12	Low grade fever	Pyrexia	10037660	General	10018065
		Right Hypochondrium pain	Pain in the upper	10000087	Gastrointestinal	10017947
		Ascites	Ascites	10003445	Gastrointestinal	10017947
Study	EC08D01	Post- surgical hemorrhagic shock	hemorrhagic shock	10049771	Vascular	10047065
		Neutropenia	Neutropenia	10029354	Blood and	10005329
		Leukocytosis and Neutrophilia	Leukocytosis	10024378	Blood and	10005329
			Neutrophilia	10029379	Blood and	10005329
	EC08D08	Hypochondrial Pain	Pain in the upper	10000087	Gastrointestinal	10017947
		Right hemithorax pain	Chest pain	10008479	General	10018065
		Vomits	Vomits	10047700	Gastrointestinal	10017947
		Vomits	Vomits	10047700	Gastrointestinal	10017947
		Epigastric pain	Pain in the upper	10000087	Gastrointestinal	10017947
		Cardial Hypotonia	Failure of	10062879	Gastrointestinal	10017947
	EC08D11	Pruritus	Pruritus	10037087	Skin and	10040785
		Post-surgical pain	Pain associated	10064882	Traumatic	10022117
		Subphrenic collection	Allocated	10066768	Gastrointestinal	10017947
		Leukocytosis	Leukocytosis	10024378	Blood and	10005329
		Leukocytosis	Leukocytosis	10024378	Blood and	10005329
		Bilateral pleural effusion	Pleural effusion	10035598	Respiratory,	10038738
		Hemodynamic instability	Hemodynamic	10052076	Vascular	10047065
		Anemia	Anemia	10002034	Blood and	10005329
	EC08D13	Vomits	Vomits	10047700	Gastrointestinal	10017947
		Abdominal pain	Abdominal pain	10000081	Gastrointestinal	10017947
		Cephaleas	Cephalea	10019211	Nervous system	10029205
		Right hemithorax pain	Chest pain	10008479	General	10018065
		Sinus tachycardia	Sinus tachycardia	10040752	Heart disorders	10007541
		Right subphrenic collection	Allocated	10066768	Gastrointestinal	10017947
		Hyperdefecation	Frequent bowel	10017367	Gastrointestinal	10017947
		Skin reaction associated to sorafenib	Skin reaction	10040914	Skin and	10040785
		Recurrent hepatocarcinoma	Recurrent liver	10073070	Benign,	10029104
		Portal Thrombosis	Thrombosis of	10036206	Hepatobiliary	10019805
		Asthenia	Asthenia	10003549	General	10018065
		Anorexia	Decreased appetite	10061428	Nutrition and	10027433
		Encephalopathy (Degree i)	Encephalopathy	10014625	Nervous system	10029205

		Petechias on thighs	Petechias	10034754	Skin and	10040785
		Pulmonary thromboembolism	Pulmonary	10037377	Respiratory,	10038738
		Diarrheas	Diarrhea	10012735	Gastrointestinal	10017947
		Ascites	Ascites	10003445	Gastrointestinal	10017947
		Anemia	Anemia	10002034	Blood and	10005329
		Right Hypochondrium pain	Pain in the upper	10000087	Gastrointestinal	10017947
		Thrombopenia	Thrombocytopenia	10043554	Blood and	10005329
		Anemia	Anemia	10002034	Blood and	10005329
		Hemorrhoidal rectorrhagia	Hemorrhoidal	10054787	Gastrointestinal	10017947

3. Serious Adevrse Events

Of the 82 AE, 7 of them were considered SAE, resulting in an 8.54% of the total. In the study group, 3 SAEs occurred (7.69%) whereas a total of 4 SAE occurred in the control group (9.30%) (Table 7). Regarding the number of patients who suffered at least one serious adverse event, results showed that there was no relation between the randomization group and the occurrence of at least one SAE (60% in the study group vs 33.3% in the control group, p = 0.377). However, concerning the severity of SAE, a significant contingency was found between the randomization group and the intensity of SAE, in such way that the SAE of moderate intensity tended to occur in the control group while those of severe intensity occurred in the study group (p = 0.008).

Nevertheless, none of the SAE was related to the use of BM-MNC.

No Unexpected Serious Adverse Events occurred.

Tabla 7. Serious post-surgical adverse events

Group	Patient	Description	Intensity	Relation	AE N° (% by patient)	AE N° (% out of the total of AEs)
Study	EC08D 01	Haemorrhagic shock	Severe	Unrelated	1 (33.3%)	1 (12.5%)
	EC08D 11	Subphrenic collection	Severe	Unrelated	1 (33.3%)	1 (12.5%)
	EC08D 13	Pulmonary thromboembolism	Severe	Unrelated	1 (33.3%)	1 (12.5%)
Control	EC08D 07	Bradycardia (Iatrogenesis by Diltiazem)	Moderate	Unrelated	1 (100%)	1 (12.5%)
	EC08D 10	Fever (after embolization)	Moderate	Unrelated	1 (33.3%)	3 (50.4%)
		Intra-abdominal biliary collection	Moderate	Unrelated	1 (33.3%)	
		Persistence of biliary collection	Moderate	Unrelated	1 (33.3%)	

4. Exitus

There were two exitus, one immediately after the hepatectomy and another one 9 months later due to disease progression, both in the study group, but both were judged unrelated to the IMP.

F. ADDITIONAL INFORMATION

1. Substantial Global Modifications

Along the study, 3 amendments were made to the initial protocol, all of them substantial modifications.

2. Global disruptions and resumptions.

They didn't occur.

3. Limitations, rating sources of potential biases and inaccuracies and warnings

Data from the trial allowed to conclude that the treatment is feasible and safe. The main limitatios of our study is the small simple, too small for the analysis of efficacy. Our trial demonstrated feasibility, safety and no inferiority to the embolization but larger randomized controlled trials are necessary to establish the effects of the BM-MNC. In addition, throughout the follow-up, missing data were found that have represented a limitation in the statistical analysis. The method for missing values treatment was listwise deletion.

The study was developed at a very early stage of research and despite having certain indications of efficacy, further studies are required in larger samples to ensure the necessary statistical power to detect significant changes over time and differences between the groups. treatment.

4. Conclusions

A Safety analysis of this intraportal route is the most clinically relevant objective in the trial, since the appearance of post-infusion portal thrombosis can lead to the death of the patient. Furthermore, the application of the treatment at the same time as the portal embolization in the contralateral portal branch could precipitate the portal thrombosis of the remaining liver. In our study, we did not find any case of portal, truncal or peripheral thrombosis, nor indirect signs of it, such as necrosis or liver abscesses on CT scan, nor enzymatic alterations in blood samples. Regarding the technique used to introduce SC inside the portal branch, in order to avoid portal thrombosis, it is very important that the catheter is insinuated in the main portal branch and not introduced into terminal branches. On the other hand, the concentration of BM-MNC is also a factor to be taken into account. An excessive number could facilitate the appearance of thrombosis. In the present safety study, an average 45.8 ± 6.57ml (35-50) containing 1460 x10^6 ± 607x 10^6 MNC were slowly infused for 15 minutes. Regarding the quality of the process the viability of BM-MNC was 96.8% (94-98%). There were two mild adverse events in a single patient that could be linked to the administration of the SC, but they actually were associated to portal embolization. These events were minor although prolonged hospital stay (5 days). We did not find liver damage neither at the tissue level nor in biochemical liver damage markers. In this study we have not observed fibrosis in the regenerated liver tissue.

An interesting aspect to note is that all the patients in the group treated with stem cells were able to receive the surgical technique planned preoperatively according to the embolized segments. The liver growth was appropriate for the proper performance of the surgical procedure, both in terms of oncological criteria and to ensure a functionally optimal remnant. The residual liver volume was higher in the study group compared to the control group, 724.4 cc vs 697.7 cc (Table 5), although the difference was not statistically significant. However, it is an important result considering the small number of patients studied. There were also no significant differences in postoperative liver function.

Taking our data together, The procedure was completed in 83.33% of patients assigned to the study group and no serious adverse events were recorded. Volumetric analyses showed no differences between the groups in the extent of regeneration that was dependent on the initial residual volume. In our experience the intraportal infusion of autologous BM-MNC in the remnant liver in addition to portal embolization appears to be a safe method that does not involve a risk to the patient, beyond that related to portal embolization itself. In spite of the interesting data founded in the present study, the sample size is too small for the analysis of efficacy, being a limitation of this study.

In conclusion, the injection of autologous bone-marrow mononuclear cells in the remaining portal branches was feasible and safe. Larger clinical trial is granted to analyze efficacy of the BM-MNC.

5. A statement by the applicant regarding the accuracy of the information submitted.

The sponsor, the Andalusian Network in Design and Translation of Advanced Therapies, through the Progreso y Salud Foundation, declares that it has monitored 100% of the information included in the Data Collection Notebooks, comparing the same with the corresponding source documents, and declares that the information presented is accurate.

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