MESENCHYMAL STEM CELLS (MSC):

A FUTURE IN HEPATOLOGY AND LIVER TRANSPLANTATION ?

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Research Article

EASL JOURNAL OF HEPATOLOGY

Infusion of mesenchymal stromal cells after deceased liver transplantation: A phase I–II, open-label, clinical study

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See Editorial, pages 7–9

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Background

- High incidence of terminal liver diseases
  - acute
  - chronic
  - genetic
  - cancerous

- Limited access to liver transplantation
- Side effects of immunosuppression

Background

- High incidence of terminal liver diseases
  - acute
  - chronic
  - genetic
  - cancerous

Artificial liver?
Cell transplantation ?

- Limited access to liver transplantation
- Side effects of immunosuppression

Hepatocyte transplantation ?

ELSEVIER

Cell Transplantation, Vol. 7, No. 4, pp. 357–363, 1998

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Original Contribution

TRANSPLANTATION OF HEPATOCYTES FOR PREVENTION OF INTRACRANIAL HYPERTENSION IN PIGS WITH ISCHEMIC LIVER FAILURE

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Hepatocyte transplantation

- Source of hepatocytes (human, animal, tumor lines)
- Culture and differentiation
- Site of injection
- Rejection (IS?)
- Monitoring

Stem cells

- Cells that can differentiate into other types of cells and can also divide in-self renewal to produce more stem-cells

- Adult stem cells act as repair system of the body

Bone Marrow stem cells

Granulocyte colony-stimulating factor and autologous CD133-positive stem-cell therapy in liver cirrhosis (REALISTIC): an open-label, randomised, controlled phase 2 trial

Philip Noel Newsome, Richard Fox, Andrew L King, Darren Barton, Nwe-Ni Than, Joanna Moore, Christopher Corbett, Sarah Townsend, James Thomas, Kathy Guo, Diana Hull, Heather A Beard, Jacqui Thompson, Anne Atkinson, Carol Bienek, Neil McGowan, Neil Guha, John Campbell, Dan Hollyman, Deborah Stocken, Christina Yap, Stuart John Forbes

Summary

Background Results of small-scale studies have suggested that stem-cell therapy is safe and effective in patients with liver cirrhosis, but no adequately powered randomised controlled trials have been done. We assessed the safety and efficacy of granulocyte colony-stimulating factor (G-CSF) and haemopoietic stem-cell infusions in patients with liver cirrhosis.

Methods This multicentre, open-label, randomised, controlled phase 2 trial was done in three UK hospitals and recruited patients with compensated liver cirrhosis and MELD scores of 11·0–15·5. Patients were randomly assigned (1:1:1) to receive standard care (control), treatment with subcutaneous G-CSF (lenograstim) 15 μg/kg for 5 days, or treatment with G-CSF for 5 days followed by leukapheresis and intravenous infusion of three doses of CD133-positive haemopoietic stem cells ($0 \cdot 2 \times 10^6$ cells per kg per infusion). Randomisation was done by Cancer Research UK Clinical Trials Unit staff with a minimisation algorithm that stratified by trial site and cause of liver disease. The coprimary outcomes were improvement in severity of liver disease (change in MELD) at 3 months and the trend of change in MELD score over time. Analyses were done in the modified intention-to-treat population, which included all patients who received at least one day of treatment. Safety was assessed on the basis of the treatment received. This trial was registered at Current Controlled Trials on Nov 18, 2009; ISRCTN, number 91288089; and the European Clinical Trials Database, number 2009-010335-41.

Interpretation G-CSF with or without haemopoietic stem-cell infusion did not improve liver dysfunction or fibrosis and might be associated with increased frequency of adverse events compared with standard care.

Liver stem cells

- Promethera

- Genetic liver diseases (Crigler Najar, Urea cycle disorders)
- Acute-on-chronic liver failure

MSC

ORIGINAL ARTICLE

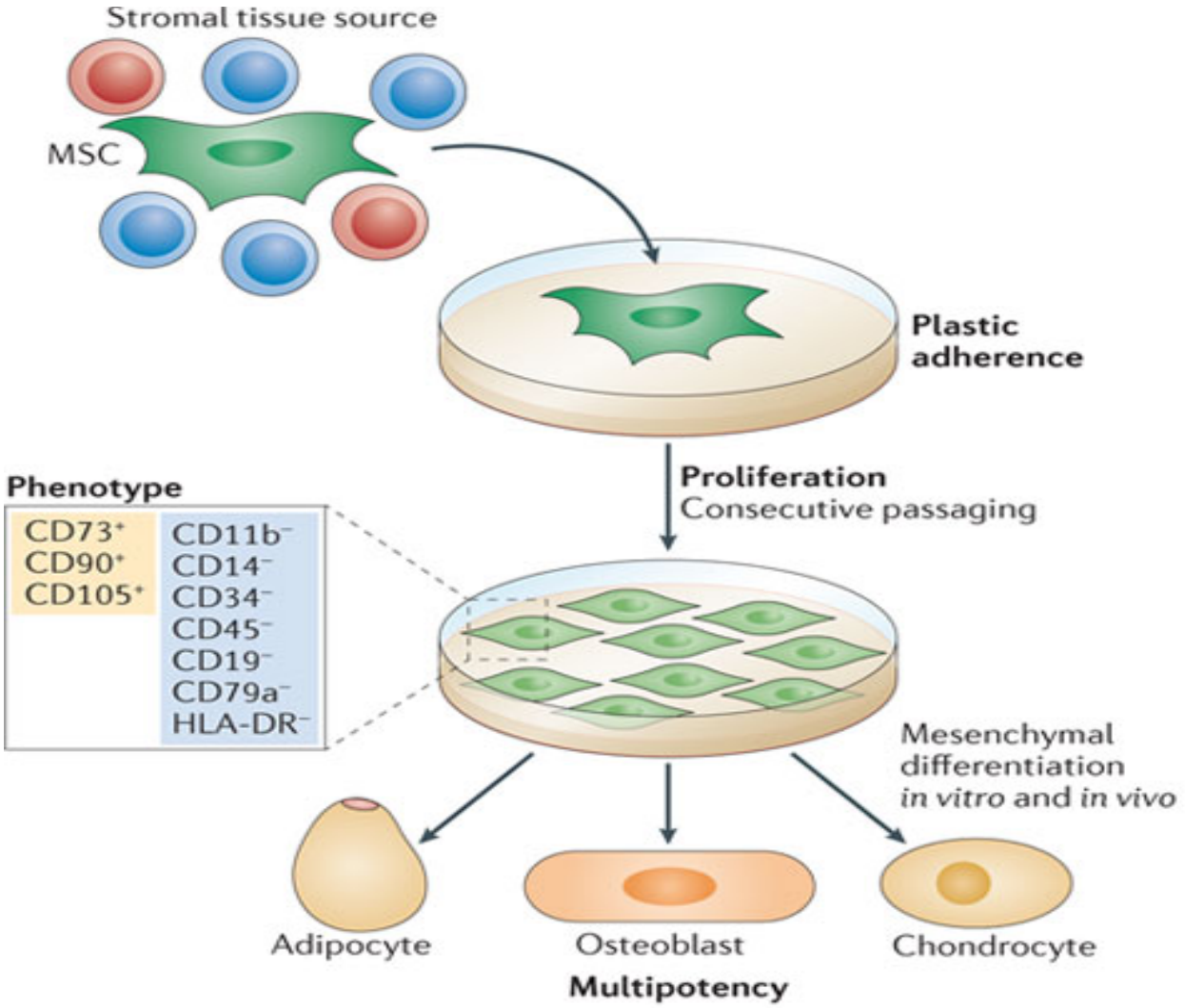
Retrospective Cohort Study

Efficacy and safety of autologous stem cell transplantation for decompensated liver cirrhosis: A retrospective cohort study

Ming-Fang Wang, You-Bing Li, Xiao-Juan Gao, Hao-Yang Zhang, Su Lin, Yue-Yong Zhu

Mesenchymal Stromal Cells (ISCT 2006)

- bone marrow
- umbilical cord
- adipose tissue
- muscle
- kidney
- …



Background

- MSC may have an immunosuppressive and/ or immunoregulatory effect

MSC are clinically used in GVHD after BM Tx

- MSC may have an anti-inflammatory effect
- MSC may have an effect in organ regeneration
- MSC may protect from I/R injury

- Role of MSC in liver diseases and in SOT?

INFUSION OF THIRD-PARTY MESENCHYMAL STROMAL CELLS (MSC) AFTER KIDNEY OR LIVER TRANSPLANTATION: A PHASE I-II, OPEN-LABEL, CLINICAL STUDY

(EudraCT 2011-001822-81 & NCT01429038)

O Detry, M Vandermeulen, MH Delbouille, J Somja, N Bletard, A Briquet, C Lechanteur, O Giet, E Baudoux, M Hannon, F Baron, Y Beguin

Dpts of Abdominal Surgery & Transplantation, Pathology, and Hematology
Laboratory of Cell and Gene Therapy (LTCG)

CHU Liège, GIGA-R, University of Liège, Belgium

Background: MSC in SOT ?

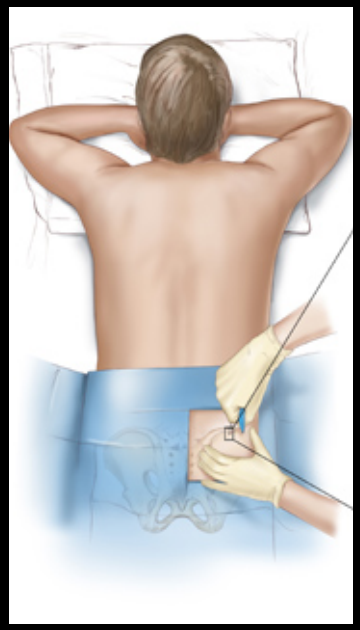
- Pulmonary embolism ?
- Cytokine-release syndrome ?
- Allergy ?
- Anti-HLA immunisation ?
- Over immunosupression ?
- Cancer ?
- Graft vascular thrombosis ?

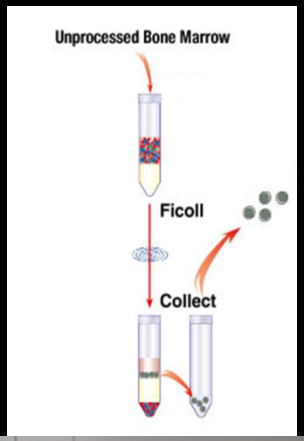
Objectives

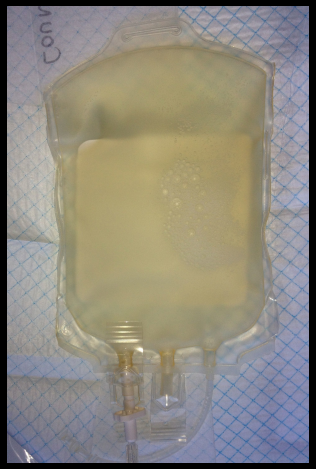
- Primary endpoint: feasibility & safety for LT recipients - tolerability of infusion
  - infections (bacterial, viral, fungi)
  - cancers (PTLD, others)
  - patient and graft survivals

- Secondary endpoint 1: immunosuppression
  - rejection rate
  - biopsy
  - blood immune profile
- Secondary endpoint 2: graft function & biopsy

MSC
Cell production







Bone marrow collection (volunteer)

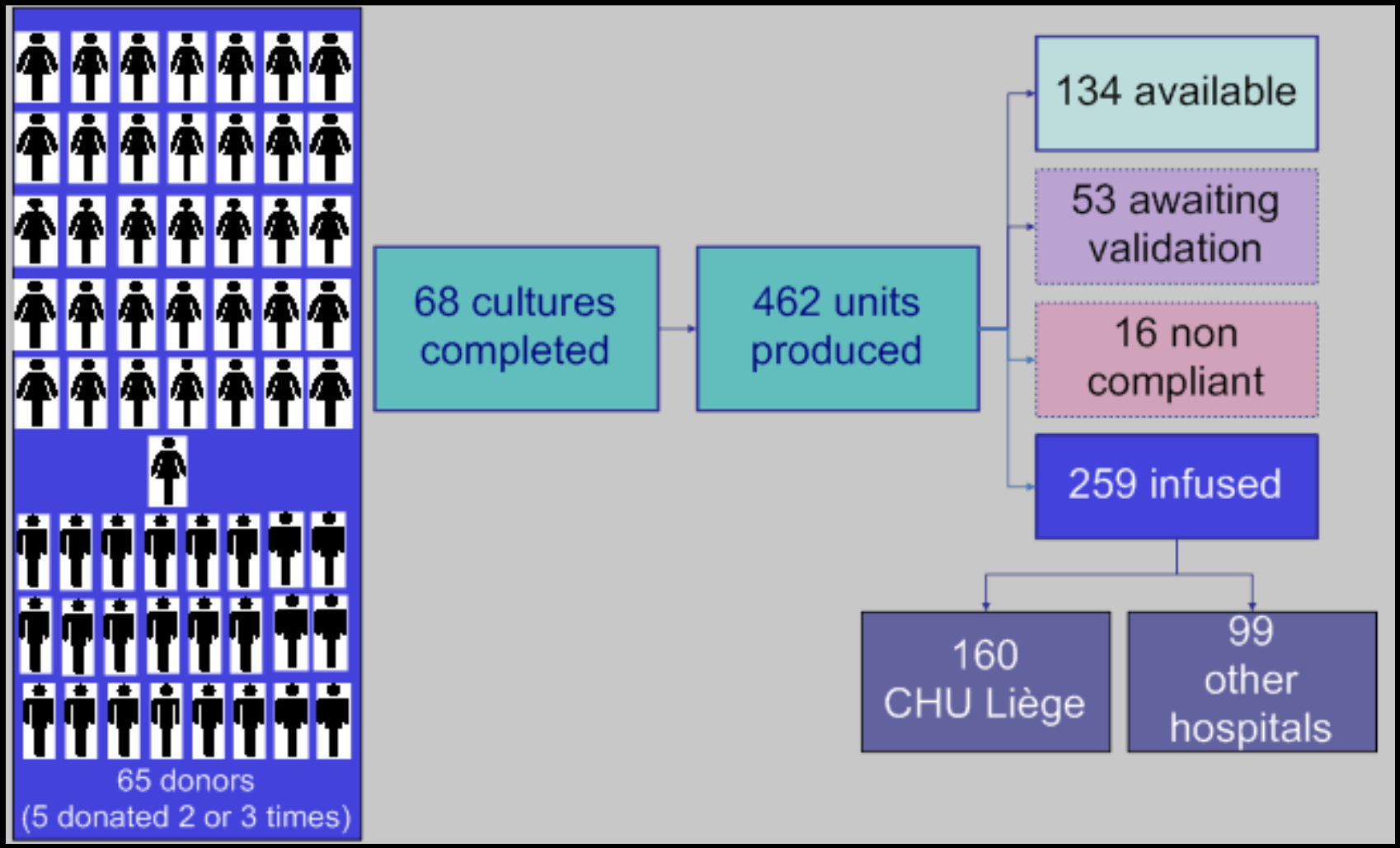
MNC isolation

Culture (4 weeks) Expansion

Freezing & banking

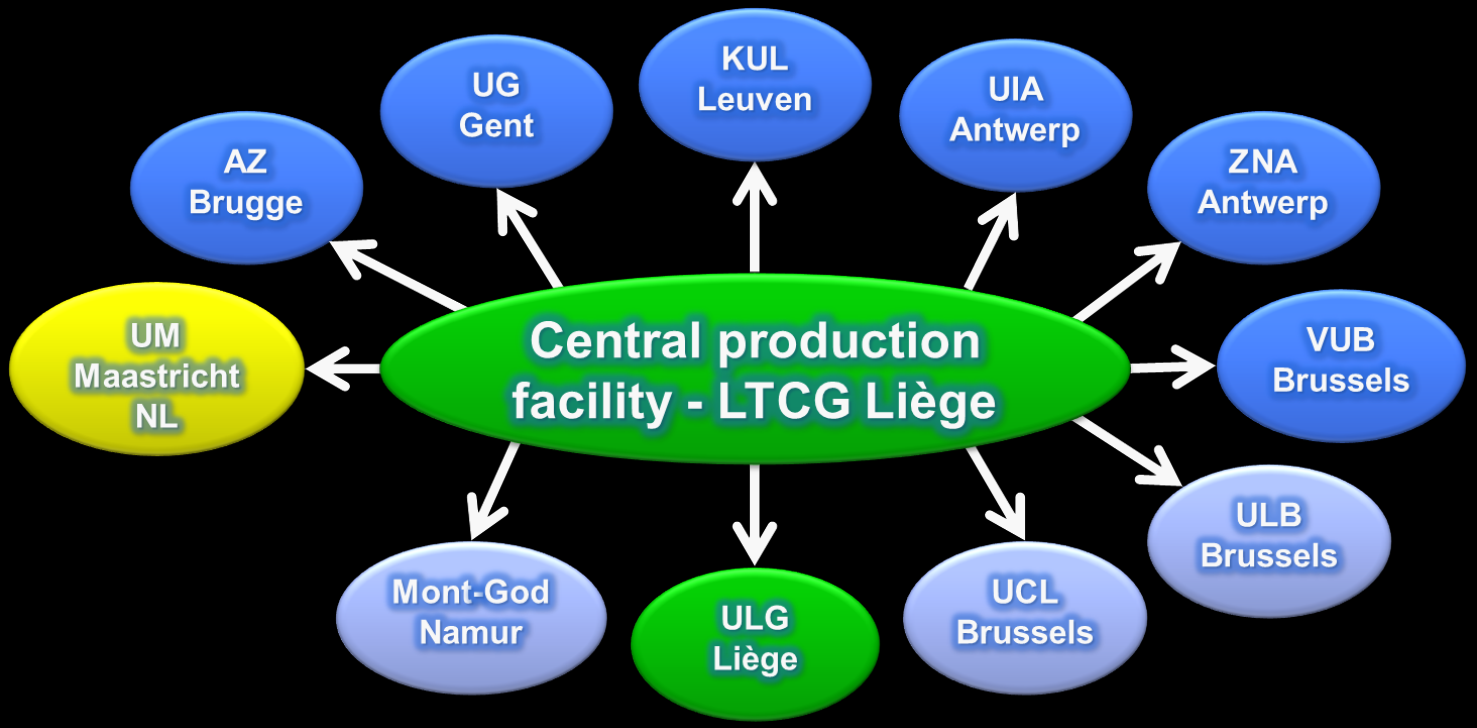
MSC

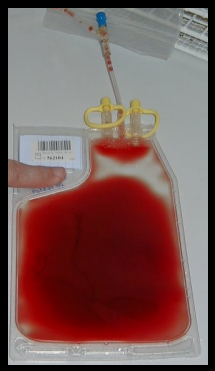
MSC bank


65 donors
(5 donated 2 or 3 times)
68 cultures completed
462 units produced
134 available
53 awaiting validation
16 non compliant
259 infused
160 CHU Liège
99 other hospitals

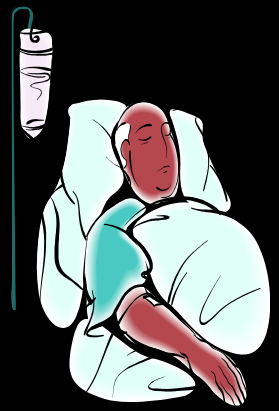
ALLOGENEIC MSC

BHS transplant clinical network





Thawing & infusion



6 clinical trials

M&M

- Cadaveric liver recipients (DBD & DCD)
- Classical immunosuppressive management

- Dose: 1.5 to 3.10^6 MSC/kg
- Central IV injection at day 3 +/-2 post Tx

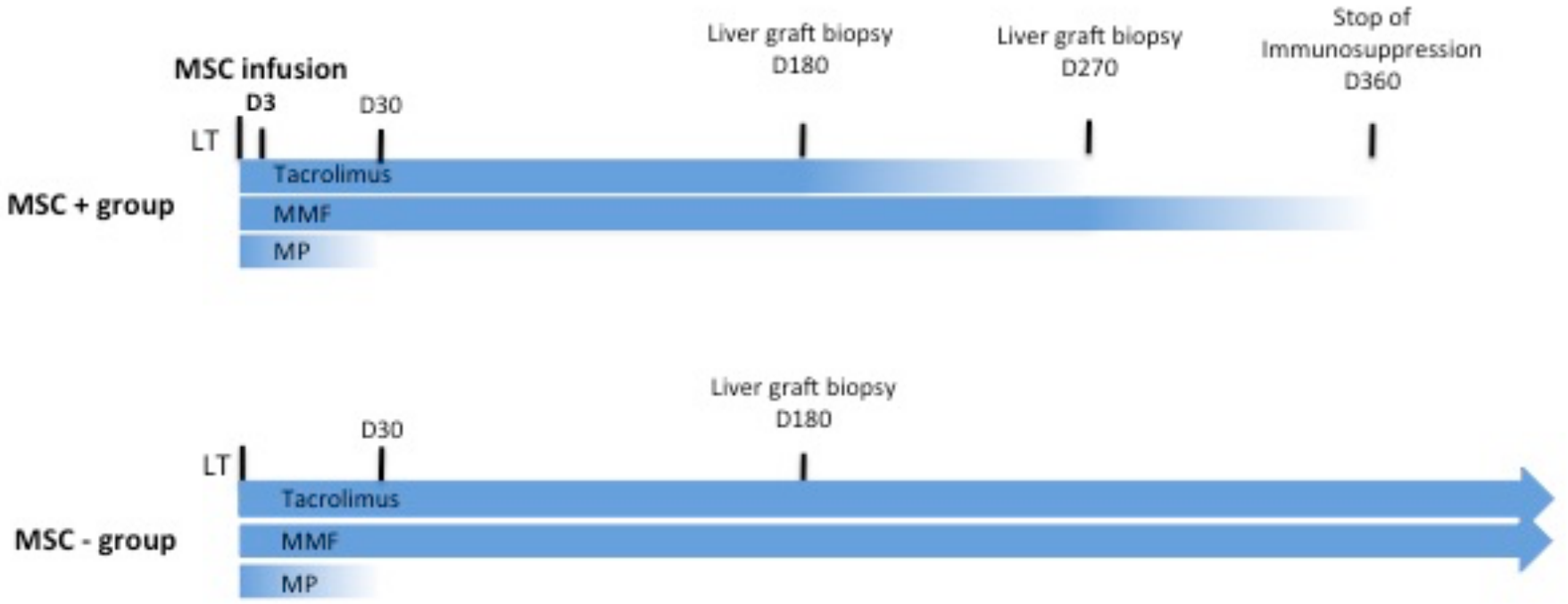
M&M: liver 1

- Liver transplantation
  - 2 groups: -10 MSC +: MSC group
    -10 MSC -: Control group

  - prospective, no randomisation, unblinded
  - regular immunosupression (TAC-MMF)
  - protocol biopsy at month 6 in both groups
  - follow-up of one year completed for all patients

- MSC group:
  - tapering of TAC from month 6 to 9, then biopsy
  - tapering MMF from month 9 to 12

M&M


MSC infusion
D3
D30
LT
Liver graft biopsy
D180
Liver graft biopsy
D270
Stop of
Immunosuppression
D360
MSC + group
Tacrolimus
MMF
MP
Liver graft biopsy
D180
D30
LT
MSC - group
Tacrolimus
MMF
MP

M&M

- Blood: FACS, Tregs, Ig, anti-HLA

- Biopsies:
  - Histology
  - Immunohistology banking of serum & biopsies

Liver recipients

		MSC+ (n=10)	MSC- (n=10)	*P*
Age (years)		62.5 (47-74)	58 (52-69)	0.516
Male/Female		8/2	7/3	1
Lab MELD		16.5 (6-29)	15 (8-38)	0.491
BMI		25.7 (20.9-38.2)	25.6 (22.2-33.0)	0.541
Liver disease				
	Post alcoholic cirrhosis	5	5	
	NASH	3	0	
	HCC	2	5	

Median (Ranges) or *n* (Mann Whitney or Fischer test

Liver donors & Transplantations

	MSC+ group (n=10)	Control group (n=10)	*P*
Age (years)	57 (17-77)	54 (18-79)	0.985
Male/Female	4/6	6/4	0.656
CPR (Y/N)	4/6	3/7	1
Donor type (DBD/DCD)	4/6	5/5	1
BMI (kg/m^2)	24 (21-31)	25 (22-29)	0.510
Intensive care stay (days)	4 (1-75)	6.5 (2-29)	0.401
Urinary output (mL/h)	82 (7-160)	127.5 (47-357)	0.096
Pressors (Y/N)	5/5	6/4	1
Na (mmol/L)	144 (133-155)	144.5 (141-160)	0.445
Total bilirubin (mg/dL)	0.35 (0.30-1.59)	0.32 (0.15-0.85)	0.668
AST (U/L)	38 (23-190)	48.5 (26-91)	0.615
GGT (U/L)	59.5 (14-256)	68 (12-144)	0.888
CIT (min)	229 (149-800)	345 (181-713)	0.386
TIT (min)	317.5 (186-831)	402.5 (216-754)	0.519

Median (Ranges) or *n* (Mann Whitney or Fischer test

MSC injection in LT recipients

	Per protocol	Study (Median) (IQR; Ranges)
MSC Injection day	day 3 +/- 2	3 (3-3.25; 2-5)
Dose MSC (10^6/kg)	1.5-3	2.1 (2.0-2.4; 1.9-2.7)
Injection volume (ml)		342 (322-469; 306-614)
Injection duration (min)		25 (16.2-40; 11-60)

Median (IQR; Ranges)

Infusional toxicity

	Pre Infusion	15 min	End of infusion	*P*
Body temperature (°C)	36.0 (35.4-37.7)	36.3 (35-36.9)	36.2 (35.5-37)	0.87
Mean PA (mmHg)	103.3 (87-124)	107 (84-119.5)	106 (94-115)	0.83
Heart rate (per min)	81 (65-102)	82.5 (65-102)	80.5 (68-101)	0.17
NI O2 saturation	99 (93-100)	100 (92-100)	97.5 (93-100)	0.67

Median (Ranges) (Friedman test & ANOVA)

- No hepatic artery or portal vein thrombosis
- No sign of pulmonary embolism or post infusional intubation
- No anaphylactic reaction, no skin reaction

Infectious complications

		MSC group (n=10)	Control group (n = 9)	P
Overall		2	6	0.06
Fungal		0	0	
Viral	CMV disease	0	0	
	HSV	2	0	
	VZV	0	1	
Bacterial	Wound	0	1	
	Urinary	0	2	
	Sinusitis	0	1	
	Pulmonary	0	1	

n (Chi square)

Cancer (6-month follow-up)

	MSC group (n=10)	Control group (n = 9)	P
Overall	1	0	>0.99
de novo	0	0	
HCC recurrence	1	0	

n (Chi square)

Follow-up at 6 months

- No rejection in either groups
- One death in the control group (hepatic artery fistula)

Month-6 graft biopsies

	MSC group (n=10)	Control group (n = 9)	P
Banff	3 (0-6)	4 (0-7)	0.21
Fibrosis	1 (0-2)	1 (0-3)	0.48

median (Ranges)

Blood tests Day 7

	MSC group (n=10)	Control group (n = 9)	P
total bilirubin (mg/L)	10.2 (4.6-26.8)	8.3 (3.7-20.7)	0.21
AST (U/L)	28.5 (19-101)	46 (30-105)	0.16
Alcaline Ph (U/L)	140 (43-475)	256 (172-590)	0.04
GGT (U/L)	218 (29-626)	368 (172-760)	0.24
INR	1.14 (1-1.21)	1.06 (1-1.26)	0.16
creatinine (mg/L)	11.55 (5.7-36)	8.9 (5.9 – 16.9)	0.32
CRP (mg/L)	32.8 (8.4-50.1)	24.6 (12.8-144.3)	0.82
tacrolimus (µg/L)	7.1 (3.1-9)	9 (2.1-11.7)	0.12

median (Ranges)

Blood tests Month 1

	MSC group (n=10)	Control group (n = 9)	P
total bilirubin (mg/L)	5.6 (3.4-11.6)	4.6 (1.3-7.5)	0.34
AST (U/L)	18 (11-51)	16 (9-61)	0.48
Alcaline Ph (U/L)	137.5 (53-554)	144 (103-857)	0.43
GGT (U/L)	101 (26-596)	112 (42-690)	0.82
INR	1.15 (0.97-1.26)	1.08 (1-1.19)	0.53
creatinine (mg/L)	16.2 (5.3-24.4)	14.1 (8.2-27.6)	0.45
CRP (mg/L)	12.9 (4.8-62.2)	17.2 (3.5-73)	0.94
tacrolimus (µg/L)	8.1 (2.4-10)	8 (5-16.3)	0.51

median (Ranges)

Blood tests Month 3

	MSC group (n=10)	Control group (n = 9)	P
total bilirubin (mg/L)	4.8 (3-19.8)	4.3 (2.3-7.5)	0.34
AST (U/L)	20 (14-31)	20 (11-58)	0.79
Alcaline Ph (U/L)	101.5 (56-1461)	119 (86-570)	0.54
GGT (U/L)	58.5 (15-695)	49 (14-332)	0.76
INR	1.1 (0.95-1.29)	1.13 (1.01-1.56)	0.65
creatinine (mg/L)	12.05 (5-25.7)	13.4 (7-21.7)	0.92
CRP (mg/L)	3.1 (1-27.6)	6.8 (1.3-23.5)	0.20
tacrolimus (µg/L)	7.7 (3.7-13)	6.4 (5.2-13.2)	0.61

median (Ranges)

Blood tests Month 6

	MSC group (n=10)	Control group (n = 9)	P
total bilirubin (mg/L)	6.6 (3.7-25.7)	4.6 (0.43-27)	0.27
AST (U/L)	25 (15-44)	24 (14-136)	0.64
Alcaline Ph (U/L)	143.5 (67-1,166)	186 (82-554)	0.26
GGT (U/L)	81 (22-978)	53 (12-2,064)	0.43
INR	1.1 (1-1.26)	1.07 (1-1.17)	0.23
creatinine (mg/L)	11.6 (7.1-18.9)	10.1 (1.28-15.8)	0.30
CRP (mg/L)	3.5 (0.7-36.5)	5.6 (0.9-151)	0.23
tacrolimus (µg/L)	4.9 (2.3-9.3)	7.4 (4.9-13)	0.02

median (Ranges)

Month-6 graft biopsies

	MSC group (n=10)	Control group (n = 9)	P
CD3	196 (95-334)	162 (93-590)	0.86
CD4	101 (54-212)	103 (17-496)	> 0.99
CD8	69 (15-196)	85 (12-300)	0.49
CD68	28.5 (12–75)	40 (15-104)	0.58
CD1a	1 (0-3)	1 (0-3)	0.83
CD138	7.5 (4-38)	6 (2-44)	0.50
CD20	27 (3-95)	28 (10-163)	0.66
FoxP3	2 (0-16)	4 (0-33)	0.49

median (Ranges)

Follow-up at 1 year

- Patient and graft survivals at 90% in both groups

Cancer

	MSC group (n=10)	Control group (n = 9)	P
Overall	1	1	>0.99
de novo	0	0	
HCC recurrence	1	1	

Peripheral Blood Lymphocyte count

Month 1	MSC group (n=10)	Control (n=9)	P
White blood cells (/µL)	6,630 (3,280-9,700)	5,190 (4,150-10,030)	0.67
Lymphocytes (/µL)	855 (380-1,690)	940 (300-1,550)	0.92
CD3 (/µL)	687 (288-1,406)	620 (200-1,336)	0.48
CD45RA (/µL)	119 (50-557)	147 (48-234)	0.70
CD45RO (/µL)	373 (179-516)	201 (79 -609)	0.23
CD3+CD4+ (/µL)	535 230-978)	349 (128-786)	0.30
CD3+CD56+ (/µL)	27 (1-87)	42 (4-154)	0.35
CD3+CD8+ (/µL)	115 (49-418)	142 (57-336)	0.76
CD19 (/µL)	144 (30-286)	99 (38-369)	0.70
CD56 (/µL)	109 (45-365)	188 (58-618)	0.27

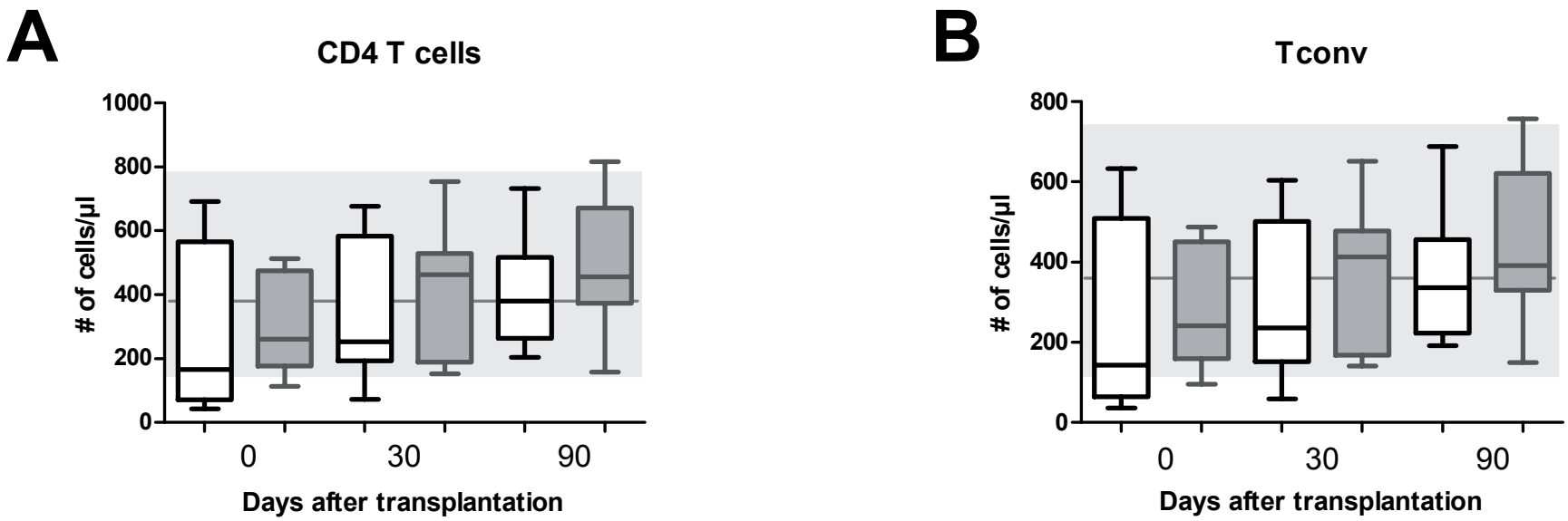
Peripheral Blood Lymphocyte count

Month 3	MSC group (n=10)	Control (n=9)	P
White blood cells (/µL)	5,265 (970-8,160)	5,200 (2,470-7,030)	0.39
Lymphocytes (/µL)	875 (420-1,880)	760 (490-1,760)	0.82
CD3 (/µL)	767 (352-1,225)	553 (274-1,419)	0.30
CD45RA (/µL)	123 (51-389)	82 (54-259)	0.58
CD45RO (/µL)	381 (171-680)	179 (135-765)	0.23
CD3+CD4+ (/µL)	516 (292-923)	285 (202-976)	0.27
CD3+CD56+ (/µL)	21 (1-99)	34 (2-197)	0.76
CD3+CD8+ (/µL)	202 (41-496)	228 (56-362)	0.94
CD19 (/µL)	93 (34-354)	100 (21-321)	0.76
CD56 (/µL)	154 (66-331)	119 (59-550)	0.82

Peripheral Blood Lymphocyte count

Month 6	MSC group (n=10)	Control (n=9)	P
White blood cells (/µL)	4,815 (4,200-8,150)	5,440 (2,680-11,430)	0.99
Lymphocytes (/µL)	1,250 (660-2,260)	1,000 (540-1,340)	0.23
CD3 (/µL)	880 (395-2,098)	592 (342-1,366)	0.27
CD45RA (/µL)	127 (76-364)	108 (61-298)	0.58
CD45RO (/µL)	396 (214-615)	267 (156-864)	0.20
CD3+CD4+ (/µL)	623 (348-728)	359 (224-1,163)	0.20
CD3+CD56+ (/µL)	31 (1-91)	36 (3-117)	0.54
CD3+CD8+ (/µL)	238 (38-1,471)	210 (73-345)	0.70
CD19 (/µL)	99 (25-256)	192 (52-258)	0.27
CD56 (/µL)	191 (66-386)	210 (55-490)	>0.99

Peripheral blood CD4+ T cells



Peripheral blood CD4+ T cells

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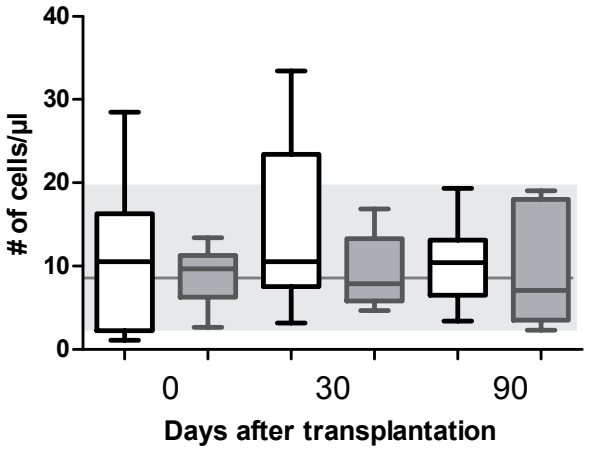

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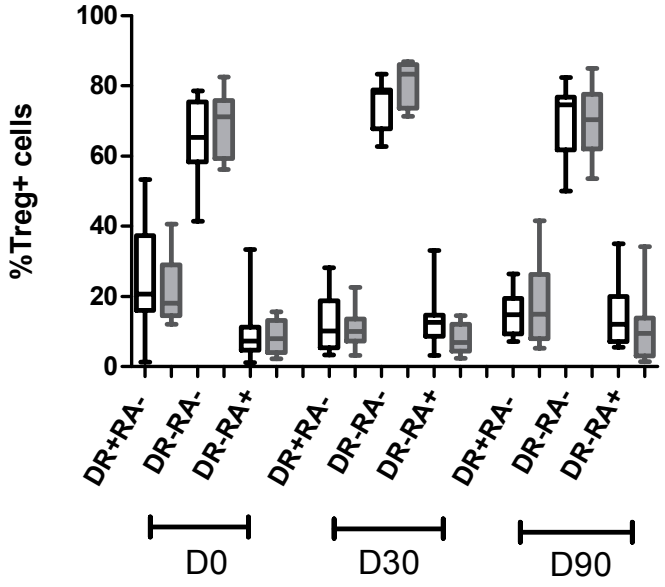

HLA

- HLA liver recipient
- HLA liver donor
- HLA MSC donor

- CDC
- Luminex

anti - HLA

- CDC ≠ Luminex

- Control group
  anti-HLA: liver donor
  anti-HLA: other – transfusion?

Phase 2 trial: IS weaning

- 9 MSC + patients underwent MSC weaning attempt (first TACRO, then MMF)

- 1 patient was successfully weaned
- 2 patients were on MMF monotherapy at month 9, but did not “tolerate” MMF weaning
- 6 patients had increasing liver tests during TACRO weaning

Conclusions

- Third party MSC infusion is feasable and seems safe in LT recipients
- Weaning of IS after one single injection of MSC in LT patients under TAC-MMF is not possible

- Timing? dose? number? source? IS?

Present & Future

- MSC Lab (Pr F Jouret, Dr M Vandermeulen)
- GMP accreditation of the MSC
- Complete results of the kidney – MSC trial

- Phase 2 trial
- Repeated allo MSC infusion in 10 liver transplants with tolerogenic IS protocol (basilixumab – everolimus – MMF – low dose steroids and progressive IS weaning

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