Induction Therapy With Autologous Mesenchymal Stem Cells in Living-Related Kidney Transplants

A Randomized Controlled Trial

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Cellules souches mésenchymateuses

- Peu nombreuses
  - Moelle osseuse
  - Tissu adipeux, placenta, liquide amniotique, poumons fœtal, peau, coeur…
- Capacité réplicative
- Pluripotentes
  - Os, cartilage, tissu adipeux, muscle, cerveau
  - Cellules pluripotentes mésenchymateuses stromales = MSCs

- Isolement lié à:
  - capacité à se lier aux surfaces plastiques
  - caractéristiques morphologiques
    - fibroblast-like
  - immunophénotype
    - Pas de marqueur spécifique
    - CD105, 73, 90, 29
  - capacité de différenciation

- A leur surface
  - Molécules d'adhésion à leur surface:
    - VCAM-1, ICAM-1, LFA
      - Interactions avec les lym T
      - Inhibition de la prolifération des lym T
  - Peu Ag HLA classe I, pas de molécules de costimulation
    - Peu immunogénique
    - Non reconnue par lym T ni NK
    - Pas de prolifération T allogénique

In vitro

- Lym T:
  - Inhibe activation et prolifération
    - Quel que soit le stimulus antigénique
  - Induction des lym T régulateurs
- Cellules dendritiques:
  - Inhibe différentiation, maturation, fonction
  - Phénotype inhibiteur
- NK:
  - Inhibe prolifération et cytotoxicité via ↓INFγ et IL2

Immunomodulateurs

- Maintien de la tolérance périphérique
- Autoimmunité

In vivo

- Babouin:
  - Prolongation de la survie d'une greffe de peau 7 à 11j post perfusion MSCs
- Souris:
  - Encéphalomyélite aigue expérimentale
  - GVH: prévention
- Rat
  - Protection contre lésions d'ischémie-reperfusion
  - Greffe foie et îlots pancréas

Hommes

- GVH aigue et chronique
  - Garçon de 9 ans, LLA
  - Greffe cellules souches, immunosuppression
  - GVH intestinale et hépatique
  - Echec bolus, PUVA, infliximab et daclizumab
  - MSCs maman
    - Perfusion 2.10^6/kg, très bien supportée
  - Bonne évolution à 1 an

Le Blanc Lancet 2004

Hommes

- Études européennes randomisées prospectives de phase III
  - Prévention rejet aigue, GVH
  - Traitement GVHA et GVHC, corticorésistante
- Etudes non randomisées:
  - SLA
  - SEP
  - AVC
  - Maladie de Crohn

- Quelle que soit la source des MSCs:
  - Donneur
    - HLA identique, haploidentique ou mismatch
  - Receveur (+ pratique greffe rénale)
  - Moelle osseuse, tissu adipeux

Mécanismes d'action

- Homing dans tissus /organes avec lésions inflammatoires
  - Immunosuppression locale
    - Indépendant de l'antigène
    - Autologue, allogénique
  - Réparation, régénération tissus
- Dans capillaires pulmonaires
- Production de facteurs solubles inhibant les lym et les CD

Immunosuppresseurs

- Avec cyclosporine A et MPA
  - Effets immunosuppresseurs synergiques
    - ↓production INF-γ
  - Réduction des doses journalières
- Meilleure combinaison inconnue

Autologous Mesenchymal Stromal Celles and Kidney Transplantation: a Pilot Study of Safety and Clinical Feasibility

- 2 transplantés rénaux, DV apparentés, CM -, pas de DSA
- 4 mois prégreffe: ponction sternale
  - MSCs autologues
- Basiliximab ou ATG, CsA, MMF, CS
- J7: MSCs 1.7 et 2.10^6/kg
- ↓CD8 mémoire,↑lym T rég

Perico et al Clin J Am Soc Nephrol 2011

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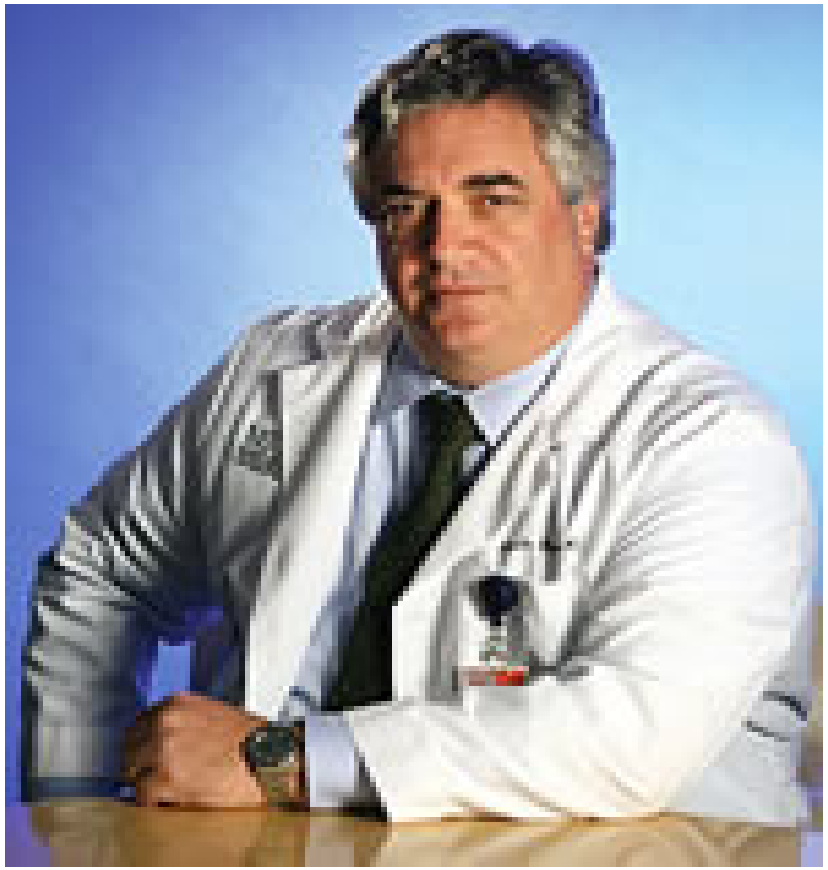
Camillo Ricordi

- Professeur chirurgie, médecine, ingénierie biomédicale, microbiologie et immunologie
- Université de Miami
- Inventeur de la machine à isoler les îlots



Fuzhou, capitale de la province du Fujian

- 1.5 millions d'habitants
- La plus grande ville chinoise proche de Taipei
- Vitrine des échanges avec Taïwan

Fuzhou General Hospital of Nanjing Military Command

Design

- Etude monocentrique, prospective, randomisée
- 02.2008-05.2009

- Profil risque-bénéfice:
  - 2 perfusions MSCs autologue
  - 2 perfusions Ac monoclonal anti-IL2 R

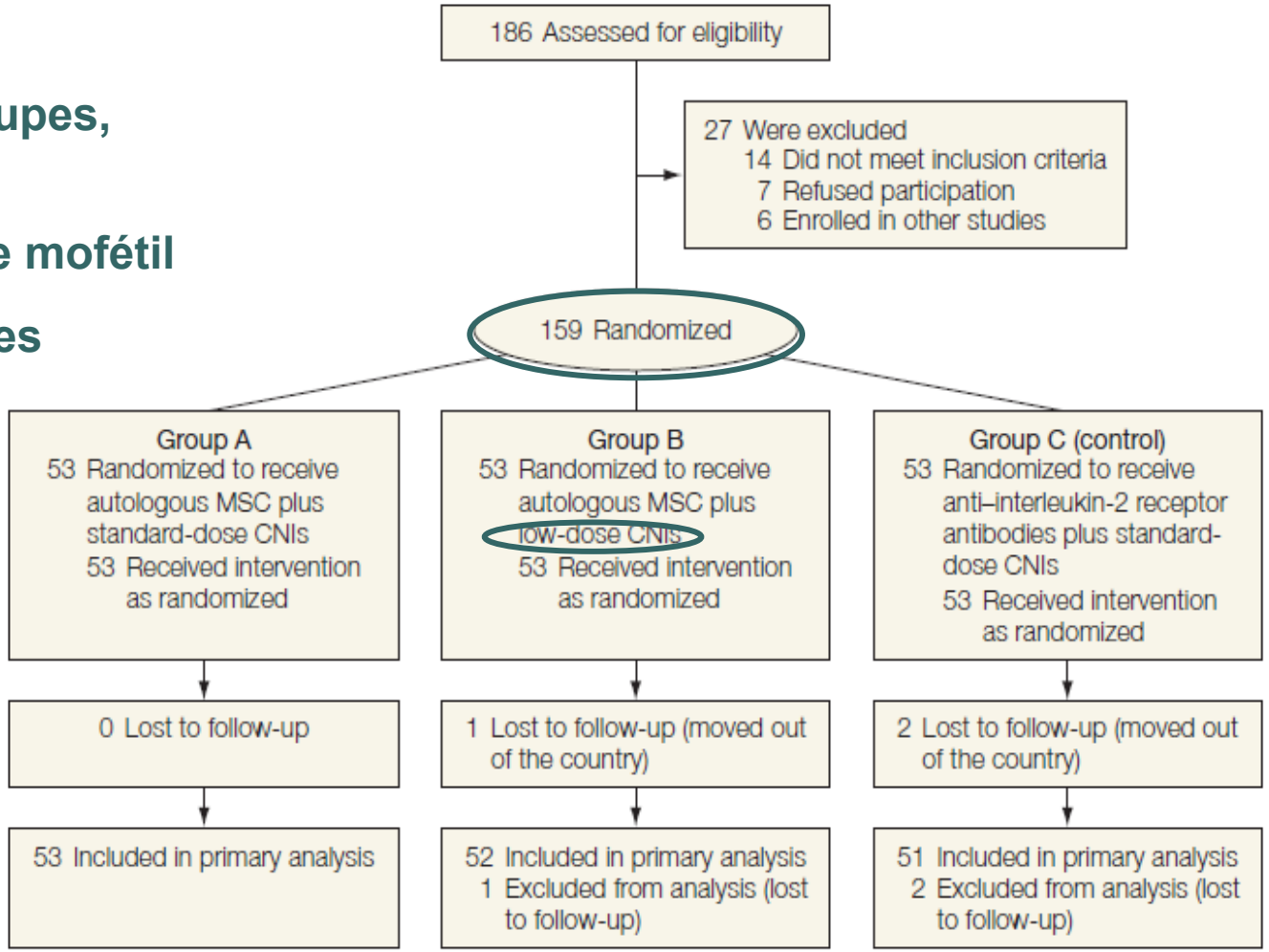
- Transplantés rénaux:
  - Faible risque immunologique
    - CM négatif, pas de DSA
    - ABO identique ou compatible
  - Donneurs vivants apparentés du 1er degré
  - Exclusions:
    - Infections systémiques, ex cancer, grossesse, BMI>28, GB<3'000, plaquettes<75'000, bilan lipidique trop perturbé

Dans les 3 groupes, mêmes doses:

Mycophénolate mofétil

Corticostéroïdes

Tac / CsA



80% dose

Culture autologue MSCs

- J-30:
  - Ponction médullaire 60-80ml, en AL, crête iliaque postérieure
- Isolement cellules mononuclées, centrifugation, adhésion à vasques avec milieux de croissance, repiquage/3j
- Phénotype caractéristique à la cytométrie de flux:CD29, CD73, CD90, CD105 +; CD34, CD45-
- Différentiation en adipocytes et ostéocytes en culture

- 70-80% confluence: cellules prélevées et remise en culture; 3-4 passages
- Testés pour les agents infectieux
- Caryotype pour exclure anomalies chromosomiques
- Perfusions:
  - pré-anastomoses vasculaires et à J15
  - 15-20mn
  - 1-2x10^6/kg

Suivi

- 1x/semaine pdt 3 mois
- 1x/mois pdt 9 mois

- Suspicion RA:
  - tjs PBR
  - bolus SM, ATG si corticorésistant

End points

- Incidence RA et eGFR à 1 an

- Survies patient et greffon à 1 an
- Incidence effets indésirables à 1 an
  - infections opportunistes

Donneurs

Age (yr)	48.9(46.1-51.7)	48.4(45.7-51.0)	49.8(47.1-52.4)	*0.926*
Gender (m/f)	28/25	27/25	28/23	*0.953*
Measured GFR (Baseline)	59.0(57.4-60.7)	60.1(58.1-62.1)	58.6(57.2-60.1)	*0.545*
eGFR (Baseline)	117.5(110.2-124.8)	119.5(113.2-125.7)	124.3(116.3-132.3)	*0.398*
eGFR (1-yr after nephrectomy)	92.7(86.3-99.0)	90.8(84.2-97.4)	91.7(86.9-96.5)	*0.901*
Baseline pathology				
Normal	39(73.6%,72.9-74.3)	39(75.0%,74.3-75.7)	37(72.5%,71.8-73.3)	*0.983*
Glomerular minimal change	6(11.3%,10.9-11.7)	7(13.5%,13.0-13.9)	5(9.8%,9.5-10.1)	
Tubular minimal change	4(7.5%,7.3-7.8)	3(5.8%,5.6-6.0)	5(9.8%,9.5-10.1)	
Others	4(7.5%,7.3-7.8)	3(5.8%,5.6-6.0)	4(7.8%,7.6-8.1)	
Gender matching (Donor/Recipient)				
Parents	35(66.0%,65.2-66.9)	33(63.5%,62.6-64.3)	31(60.8%,59.9-61.7)	*0.857*
Male (D)/Male (R)	15(28.3%,27.6-29.1)	12(23.1%,22.4-23.7)	16(31.4%,30.6-32.2)	*0.804*
Male (D)/Female (R)	5(9.4%,9.1-9.7)	8(15.4%,14.9-15.9)	5(9.8%,9.5-10.1)	
Female (D)/Male (R)	4(7.5%,7.3-7.8)	5(9.6%,9.3-9.9)	4(7.8%,7.6-8.1)	
Female (D)/Female (R)	11(20.8%,20.1-21.4)	8(15.4%,14.9-15.9)	6(11.8%,11.4-12.1)	
Brother or Sister	18(34.0%,33.1-34.8)	19(36.5%,35.7-37.4)	20(39.2%,38.3-40.1)	*0.857*
Male (D)/Male (R)	5(9.4%,9.1-9.7)	4(7.7%,7.4-8.0)	4(7.8%,7.6-8.1)	*0.804*
Male (D)/Female (R)	3(5.7%5.5-5.9)	3(5.8%,5.6-6.0)	3(5.9%,5.7-6.1)	
Female (D)/Male (R)	3(5.7%5.5-5.9)	3(5.8%,5.6-6.0)	4(7.8%,7.6-8.1)	
Female (D)/Female (R)	7(13.2%,12.8-13.6)	9(17.3%,16.8-17.8)	9 (17.6%,17.1-18.2)	
HLA mismatching**	2.7(2.5-2.9)	2.6(2.4-2.8)	2.6(2.3-2.8)	*0.946*

Receveurs

Age (yr)	39.2(36.5-42.0)*	36.8(33.9-39.8)	37.0(34.0-39.9)	*0.371*
Gender (m/f)	31/22	35/17	34/17	*0.576*
PRA positive (%)	7(13.2%,12.8-13.6)	5(9.6%,9.3-9.9)	6(11.8%,11.4-12.1)	*0.846*
Weight (kg)	58.9(56.4-61.4)	60.2(57.3-61.2)	58.5(54.9-62.1)	*0.585*
Height (cm)	165.8(163.8-167.8)	166.7(164.5-168.9)	166.2(164.2-168.2)	*0.832*
Body-mass index (kg/m^2)	21.4(20.6-22.1)	21.6(20.8-22.5)	21.2(19.9-22.6)	*0.522*
Cause of ESRD				
Hypertension	3(5.7%,5.5-5.9)	2(3.8%,3.7-4.0)	2(3.9%,3.8-4.1)	*0.965*
Diabetes mellitus	5(9.4%,9.1-9.7)	4(7.7%,7.4-8.0)	3(5.9%,5.7-6.1)	
Glomerulonephritis/vasculitis	34(64.2%,63.3-65.0)	35(67.3%,66.5-68.1)	33(64.7%,63.9-65.6)	
Polycystic kidney disease	3(5.7%,5.5-5.9)	2(3.8%,3.7-4.0)	1(1.9%,1.9-2.0)	
Obstructive uropathy	2(3.8%,3.6-3.9)	3(5.8%,5.6-6.0)	2(3.9%,3.8-4.1)	
Unknown	6(11.3%,10.9-11.7)	6(11.5%,11.2-11.9)	10(19.6%,19.0-20.2)	
Current dialysis				
Hemodialysis	39(73.6%,72.9-74.3)	36(69.2%,68.4-70.0)	36(70.6%,69.8-71.4)	*0.881*
Peritoneal dialysis	14(26.4%,25.7-27.1)	16(30.8%,30.0-31.6)	15(29.4%,28.6-30.2)	
Dialysis time (months)	6.2(5.3-7.0)	7.1(6.0-8.3)	6.5(5.7-7.3)	*0.788*
Cold ischemia time (minutes)	115.7(107.6-123.7)	116.4(107.0-125.8)	120.3(112.0-128.6)	*0.908*
Warm ischemia time (minutes)	2.1(1.8-2.3)	2.2(2.0-2.5)	2.2(2.0-2.5)	*0.932*
Operation time (minutes)	142.6(133.4-151.8)	146.2(138.7-153.7)	142.9(133.9-152.0)	*0.941*
Cytomegalovirus status				
D+/R-	1(1.9%,1.8-2.0)	2(3.8%,3.7-4.0)	2(3.9%,3.8-4.1)	*0.584*
D-/R-	52(98.1%,98.0-98.2)	50(96.2%,96.0-96.3)	49(96.1%,95.9-96.2)	
Repeated transplantation	2(3.8%,3.6-3.9)	2(3.8%,3.7-4.0)	3(5.9%,5.7-6.1)	*0.842*
Comorbidities				
Hypertension	29(54.7%,53.8-55.6)	32(61.5%,60.7-62.4)	31(60.8%,59.9-61.7)	*0.738*
Diabetes mellitus	5(9.4%,9.1-9.7)	4(7.7%,7.4-8.0)	3(5.9%,5.7-6.1)	*0.794*
Hyperlypidemia	18(34.0%,33.1-34.8)	19(36.5%,35.7-37.4)	16(31.4%,30.6-32.2)	*0.858*

Primary end-point

End Point	Autologous Mesenchymal Stem Cell Treatment: Standard-Dose CNI (n = 53)	Autologous Mesenchymal Stem Cell Treatment: Low-Dose CNI (n = 52)	Control (n = 51)	*P* Value Overall Type 3[b]
Primary end point				
eGFR, mean (95% CI), mL/min per 1.73 m^2,[c]				
Posttransplant				
0 d	6.8 (4.7-8.8)	5.3 (3.1-7.6)	5.8 (3.0-8.6)	.56
7 d	77.0 (67.4-86.6)[d]	74.9 (66.3-83.6)[d]	52.6 (44.5-60.7)	<.001
14 d	84.9 (75.2-94.6)[e]	77.8 (69.0-86.6)	69.6 (61.0-78.3)	.07
1 mo	91.1 (83.7-98.4)[f]	81.4 (73.8-89.0)	79.0 (69.9-88.1)	.08
2 mo	90.1 (84.3-96.0)	85.6 (79.9-91.3)	82.3 (74.1-90.5)	.28
3 mo	88.9 (82.8-95.0)	87.9 (80.5-95.3)	85.8 (78.8-92.9)	.81
6 mo	90.6 (84.2-97.1)	82.7 (76.6-88.8)	89.4 (83.0-95.9)	.62
12 mo	93.2 (86.2-100.2)	86.7 (79.0-94.3)	85.5 (78.2-92.9)	.49

Time Point, d	eGFR Difference (95% CI), mL/min per 1.73 m²	*P* Value[a]
Autologous MSC + Standard-Dose CNI vs Control Group		
0	1.0 (–2.0 to 4.0)	.51
7	24.4 (11.9 to 37.0)	<.001
14	15.3 (2.3 to 28.3)	.02
30	12.1 (0.3 to 23.8)	.045
60	7.8 (–2.2 to 17.8)	.13
90	3.1 (–6.3 to 12.4)	.52
180	1.2 (–7.9 to 10.3)	.80
360	7.7 (–2.4 to 17.8)	.14
7-30[b]	6.2 (0.4 to 11.9)	.04
0 – 360[b]	9.1 (1.6 to 16.5)	.02
Autologous MSC + Low-Dose CNI vs Control Group		
0	–0.5 (–3.6 to 2.7)	.78
7	22.4 (10.8 to 34.0)	<.001
14	8.2 (–3.9 to 20.3)	.18
30	2.4 (–9.3 to 14.1)	.69
60	3.3 (–6.5 to 13.0)	.51
90	2.1 (–8.0 to 12.1)	.69
180	–6.7 (–15.4 to 2.0)	.13
360	1.1 (–9.3 to 11.6)	.83
7-30[b]	10.0 (3.8 to 16.2)	.002
0-360[b]	4.0 (–2.9 to 10.9)	.25
Autologous MSC + Standard-Dose vs Low-Dose CNI		
0	1.5 (–1.3 to 4.2)	.30
7	2.1 (–10.7 to 14.8)	.75
14	7.1 (–5.8 to 20.0)	.28
30	9.7 (–0.7 to 20.1)	.07
60	4.6 (–3.4 to 12.6)	.26
90	1.0 (–8.5 to 10.5)	.84
180	7.9 (–0.7 to 16.5)	.07
360	6.5 (–3.7 to 16.7)	.21
7-30[b]	–3.8 (–9.4 to 1.8)	.19
0-360[b]	5.0 (–1.8 to 11.9)	.15

Primary end-point

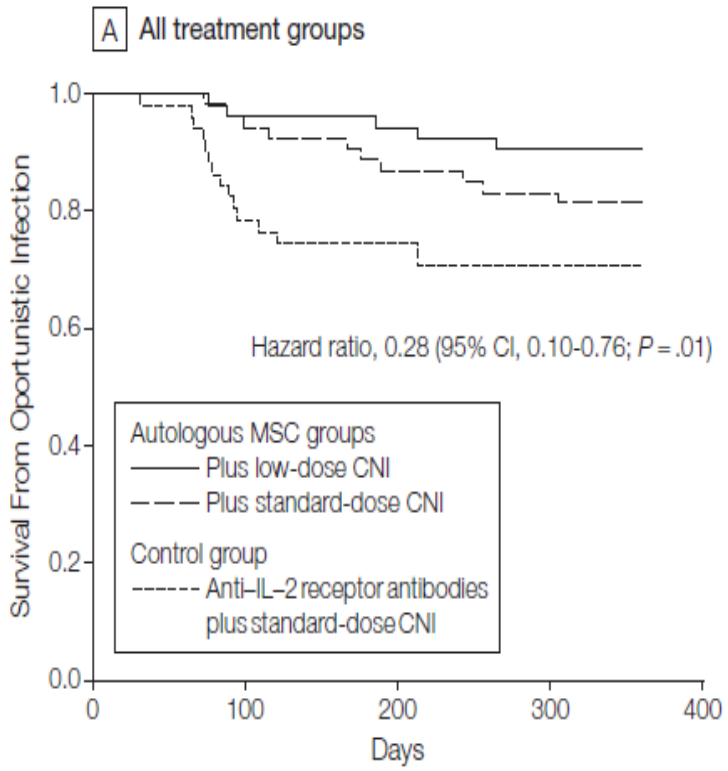
End Point	Autologous Mesenchymal Stem Cell Treatment: Standard-Dose CNI (n = 53)	Autologous Mesenchymal Stem Cell Treatment: Low-Dose CNI (n = 52)	Control (n = 51)	*P* Value Overall Type 3[b]
Acute rejection, No. (%) [95% CI]				
At 6 mo				
Biopsy-confirmed	4 (7.5) [0.4-14.7][g]	4 (7.7) [0.5-14.9][h]	11 (21.6) [10.5-32.6]	.02
Corticosteroid-resistant	0	0	4 (7.8) [0.6-15.1]	
Histological severity				
Banff I/II	4 (7.5) [0.4-14.7]	4 (7.7) [0.5-14.9]	7 (13.7) [4.5-23.0]	.007
Banff III	0	0	4 (7.8) [0.6-15.1]	
At 12 mo				
Biopsy-confirmed	8 (15.1) [5.5-24.7]	9 (17.3) [7.1-27.5]	13 (25.5) [13.8-37.2]	.37
Corticosteroid-resistant	0	1 (1.9) [0-5.6]	4 (7.8) [0.6-15.1]	.06
Histological severity				
Banff I/ II	8 (15.1) [5.5-24.7]	8 (15.4) [5.7-25.1]	7 (13.7) [4.5-23.0]	.07
Banff III	0	1 (1.9) [0-5.6]	4 (7.8) [0.6-15.1]	

Secondary end-points

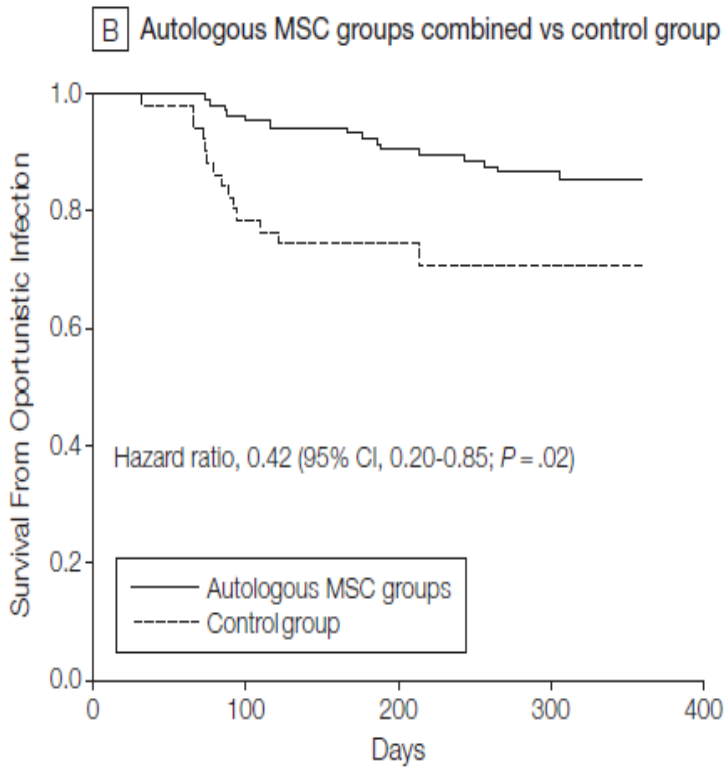
End Point	Autologous Mesenchymal Stem Cell Treatment		Control (n = 51)	*P* Value Overall Type 3[b]
	Standard-Dose CNI (n = 53)	Low-Dose CNI (n = 52)		
Secondary, No. (%) [95% CI]				
Delayed graft function	5 (9.4) [1.6-17.3]	4 (7.7) [0.5-14.9]	4 (7.8) [0.6-15.1]	.94
Duration of dialysis, mean (range), d	17.4 (10.5-24.3)	15.3 (7.9-23.1)	16.3 (10.0-22.5)	.28
Graft loss	1 (1.9) [0-5.5]	2 (3.8) [0-9.0]	1 (2.0) [0-5.7]	.85
Acute rejection	0	1 (1.9) [0-5.6]	1 (2.0) [0-5.7]	.85
Chronic rejection	1 (1.9) [0-5.5]	1 (1.9) [0-5.6]	0	
Death	0	0	0	

Pas de différence entre les 3 groupes

Events	No. (%) of Patients [95% CI]			*P* Value Overall Type 3
	Autologous Mesenchymal Stem Cell Treatment			
	Standard-Dose CNI (n = 53)	Low-Dose CNI (n = 52)	Control Group (n = 51)	
Total adverse events	35 (66.0) [53.3-78.8][b]	32 (61.5) [48.4-74.6][c]	43 (84.3) [74.5-94.1]	.01
Leukopenia				
7 d	6 (11.3) [2.8-19.9]	5 (9.6) [1.7-17.6]	4 [0.6-15.1]	.80
14 d	5 (9.4) [1.6-17.3]	6 (11.5) [2.9-20.1]	3 (5.9) [0-12.2]	.60
1 mo	3 (5.7) [0-11.9]	4 (7.7) [0.5-14.9]	2 (3.9) [0-9.1]	.71
3 mo	2 (3.8) [0-8.9]	1 (1.9) [0-5.6]	2 (3.9) [0-9.1]	.81
12 mo	0	0	1 (2.0) [0-5.7]	.36
Lymphopenia				
7 d	5 (9.4) [1.6-17.3]	5 (9.6) [1.7-17.6]	3 (5.9) [0-12.2]	.74
14 d	8 (15.1) [5.5-24.7]	7 (13.5) [4.3-22.7]	5 (9.8) [1.8-17.8]	.71
1 mo	4 (7.5) [0.4-14.7]	6 (11.5) [2.9-20.1]	4 (7.8) [0.6-15.1]	.73
3 mo	2 (3.8) [0-8.9]	1 (1.9) [0-5.6]	1 (2.0) [0-5.7]	.79
12 mo	0	0	0	>.99
All infections	28 (52.8) [39.4-66.3]	20 (38.5) [25.4-51.6]	31 (60.8) [47.6-73.9]	.07
Opportunistic infection	10 (18.9) [8.3-29.4][d,e]	5 (9.6) [1.7-17.6][f]	15 (29.4) [17.1-41.7]	.03
Candida	2 (3.8) [0-8.9]	1 (1.9) [0-5.6]	3 (5.9) [0-12.2]	
Cytomegalovirus	2 (3.8) [0-8.9]	1 (1.9) [0-5.6]	3 (5.9) [0-12.2]	
EB virus	3 (5.7) [0-11.9]	1 (1.9) [0-5.6]	5 (9.8) [1.8-17.8]	
Herpes simplex virus	3 (5.7) [0-11.9]	2 (3.8) [0-9.0]	4 (7.8) [0.6-15.1]	
Time to first opportunistic infection, HR vs control group[h]	0.6 (0.25-1.24)[g]	0.28 (0.10-0.76)		.04
Other infections	18 (34.0) [21.2-46.7]	15 (28.8) [16.6-41.0]	16 (31.4) [18.9-43.9]	.85
Nasopharyngitis	6 (11.3) [2.9-19.9]	4 (7.7) [0.5-14.9]	6 (11.8) [3.1-20.4]	
Pneumonia	4 (7.5) [0.4-14.7]	2 (3.8) [0-9.0]	4 (7.8) [0.6-15.1]	
Urinary tract infection	5 (9.4) [1.6-17.3]	6 (11.5) [2.9-20.1]	4 (7.8) [0.6-15.1]	
Phlebitis	3 (5.7) [0-11.9]	3 (5.8) [0-12.0]	2 (3.9) [0-9.1]	
Hematuria	2 (3.8) [0-8.9]	3 (5.8) [0-12.0]	4 (7.8) [0.6-15.1]	.67
Proteinuria	2 (3.8) [0-8.9]	2 (3.8) [0-9.0]	3 (5.9) [0-12.2]	.84
Complications of transplanted kidney	2 (3.8) [0-8.9]	1 (1.9) [0-5.6]	1 (2.0) [0-5.7]	.79
Delayed wound healing at 2 wk	1 (1.9) [0-5.5]	0	2 (3.9) [0-9.1]	.35
Lymphocele	1 (1.9) [0-5.5]	1 (1.9) [0-5.6]	3 (5.9) [0-12.2]	.42



No. at risk				
Autologous MSC groups				
Low-dose CNI	52	50	49	47
Standard-dose CNI	53	50	46	44
Control group	51	40	38	36



No. at risk				
Autologous MSC groups	105	100	95	91
Control group	51	40	38	36

Thérapie cellulaire

- 1ère utilisation de MSCs en phase d'induction
- Remplacement basiliximab
  - Moins de RA à 6 mois
  - Pas de RA corticorésistant
  - Récupération fonction greffon plus rapide J30
  - Moins d'infections opportunistes
- Low dose CNI
- Diminution des lésions ischémie-reperfusion
  - Effet antioxydant des MSCs dans modèle rat

Zhou et al Urol Int 2011

- Sureté à long terme?
- Follow up après 1 an
  - Infections, tumeur
- Diminution plus grande des CNI

Transplantation d'organe solide

- Janvier 2010, Rotterdam
- MSCs = candidat idéal pour le thérapie cellulaire