Predictive factors and impact of full donor T-cell chimerism after reduced intensity conditioning allogeneic stem cell transplantation

This study investigated the kinetics of CD3+-T cell chimerism (TCC) in 102 patients receiving reduced intensity conditioning allogeneic stem cell transplantation (RIC-allo-SCT) from an HLA-identical sibling. Patients with full donor TCC at day 30 had a higher incidence of grade 2-4 acute GVHD compared to patients in mixed TCC (cumulative-incidence, 61% vs. 35%; p=0.01). The delayed establishment of full donor TCC in myeloid malignancies was associated with a higher incidence of relapse (40% vs. 0; p=0.002), suggesting that monitoring of the kinetics of TCC is mandatory after RIC-allo-SCT.

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Initial mixed donor/host chimerism has usually been observed in most patients after reduced-intensity conditioning allogeneic stem cell transplantation (RIC-allo-SCT). This retrospective single center study investigated the impact of different factors on the establishment of full donor $CD3^+$ T cell chimerism (TCC) in a series of 102 patients receiving RIC allo-SCT. Only patients who were alive at the third month after RIC-allo-SCT were included. All donors were HLA-A-, HLA-B-, and HLA-DR-identical siblings. Eligibility criteria for RIC allo-SCT are detailed elsewhere.[1] The RIC regimen included either fludarabine, oral busulfan and low dose anti-thymocyte globulin (ATG 2.5 or 5 mg/kg total dose; Thymoglobulin, Genzyme, Lyon, France),[1] or fludarabine (150 mg/m^2 total dose) and low dose irradiation [TBI 2 Gy, or total lymphoid irradiation (TLI), 1.5 Gy total dose] with or without busulfan. Busulfan total dose was 8 mg/kg when ATG was used and 4 mg/kg with TLI. Choice of the RIC regimen type was not based on disease category. Supportive care has been previously reported and was similar during the whole study period.[2] Graft-vs.-host disease (GVHD) prophylaxis was given with cyclosporine A (CSA) alone or with CSA and mycophenolate mofetil (MMF). According to protocols, MMF was discontinued at day 35 after allo-SCT. CSA tapering was started between day 80 and 100 if no GVHD appeared. All patients received peripheral blood stem cells mobilized with G-CSF.

TCC (on sorted peripheral blood T lymphocytes) was serially assessed approximately 30, 60 and 90 days after allo-SCT as previously described.[3] Mixed T-cell chimerism was defined as between 5 and 94% recipient cells, and full chimerism was defined as the presence of more than 95% donor cells.[4] Patients and RIC-allo-SCT characteristics are detailed in Table 1. Kinetics of full donor TCC are shown in Figure 1A. In univariate analysis, none of the patients' graft, RIC type, or disease characteristics were predictive of establishment of an *early* full donor TCC at day 30. However, the 31 patients who achieved this experienced a higher incidence of grade 2-4 acute GVHD compared to the 71 patients who were still in mixed TCC at day 30 (cumulative incidence, 61% *vs.* 35%; p=0.01; Figure 1B). Univariate analysis of predictive factors for full donor TCC at day 90 is shown in Table 1. Diagnosis category (Figure 1C), the RIC regimen type (Figure 1D), a female donor, $CD34^+$ stem cell dose, and $CD4^+$ T cell dose infused with the graft, were significantly or had a trend towards significant association with full donor TCC at day 90. In the mul-

Table 1. Univariate analysis of risk factors for full donor T-cell chimerism at day 90 after transplantation

	Full donor chimerism n=79 (%)	*Mixed chimerism n=23 (%)*	*p*
Patient age y., median (range)	49 (18-67)	51 (34-65)	0.35
Donor age y., median (range)	48 (21-77)	48 (26-72)	0.80
Female donor	38 (48)	6 (26)	0.061
CMV serologic status, seronegative pair	12 (15)	3 (13)	0.94
ABO mismatch	48 (61)	17 (74)	0.25
Diagnosis			
Myeloid malignancy[a]	26 (33)	15 (65)	0.014
Lymphoid malignancy[b]	36 (46)	7 (30)	
Metastatic non-hematological malignancy[c]	17 (22)	1 (4)	
Disease status[d]			
Standard risk	12 (15)	7 (30)	0.18
High risk	67 (85)	16 (70)	
Conditioning regimen[e]			
ATG-based regimen	48 (61)	17 (74)	0.043
Low dose TBI-based regimen	9 (11)	5 (22)	
Low dose TLI-based regimen	22 (28)	1 (4)	
GVHD prophylaxis[f]			
CSA alone	41 (52)	14 (61)	0.45
CSA + MMF	38 (48)	9 (39)	
Graft composition (cells ($\times 10^6$/kg recipient body weight), median (range)			
CD34+ stem cells	5.4 (1.4-37)	6.5 (1.6-12.9)	0.067
CD3+ T cells	309 (84-689)	309 (84-689)	0.47
CD4+ T cells	223 (63-453)	189 (97-363)	0.082
CD8+ T cells	106 (26-323)	102 (51-255)	0.81
CD56+ T cells	36 (4-115)	31 (13-103)	0.29
CD19+ T cells	71 (14-946)	68 (27-117)	0.86
ANC > 500 /μL	18 (0-28)	17 (8-28)	0.31

Variables with p<0.10 in univariate analysis were included in the multivariate analysis. [a]Full donor chimerism group: 15 acute myeloid leukemia (AML), 5 chronic myeloid leukemia (CML), 5 myelodysplastic syndromes (MDS), and 1 myeloproliferative disorder. Mixed chimerism group: 9 AML, 2 CML, 2 MDS, 1 myeloproliferative disorder, and 1 other myeloid disorder. [b]Full donor chimerism group: 15 multiple myeloma, 15 non-Hodgkin's lymphoma, 3 chronic lymphocytic leukemia, and 3 other. Mixed chimerism group: 5 multiple myeloma, 2 non-Hodgkin's lymphoma. [c]Full donor chimerism group: 6 breast cancers, 3 renal carcinomas, 2 ovarian carcinomas, 6 other. Mixed chimerism group: 1 renal carcinoma. [d]Standard risk disease: chronic myeloid leukemia in chronic phase, acute leukemias in first complete remission. All other diseases were considered as high risk. [e]ATG-based regimen: fludarabine 150 mg/m^2, busulfan 8 mg/kg, and ATG 2.5 or 5 mg/kg; low dose TBI-based regimen: fludarabine 90 mg/m^2 and total body irradiation 2 Gy; low dose TLI-based regimen: fludarabine 150 mg/m^2, busulfan 4 mg/kg, and total lymphoid irradiation (TLI) 1.5 Gy. TLI was performed after virtual simulation and 3D treatment planning using conformational external beam radiotherapy techniques. A total dose of 1.5 Gray was prescribed to the ICRU reference point, and was delivered in a single fraction, using two AP high energy photon beams (6, 15, and/or 18 MV). The plan was optimized to maximize the dose to the planning target volume and to limit the dose to normal tissue, and a beam's-eye-view display was used to ensure optimal quality of treatment. Beam shaping was performed using custom blocks to spare normal tissues. [f]Per protocols, MMF was discontinued at day 35 after allo-SCT. CSA tapering was started between day 80 and 100 if no GVHD appeared. Only 2 patients in this series received donor lymphocyte infusions (DLI) at day 56 and day 79 after allo-SCT respectively, but DLI did not subsequently modify the TCC status of the patients. GVHD: graft-vs.-host disease; M: male; F: female; CMV: cytomegalovirus; ATG: anti-thymocyte globulin; CSA: cyclosporine A.

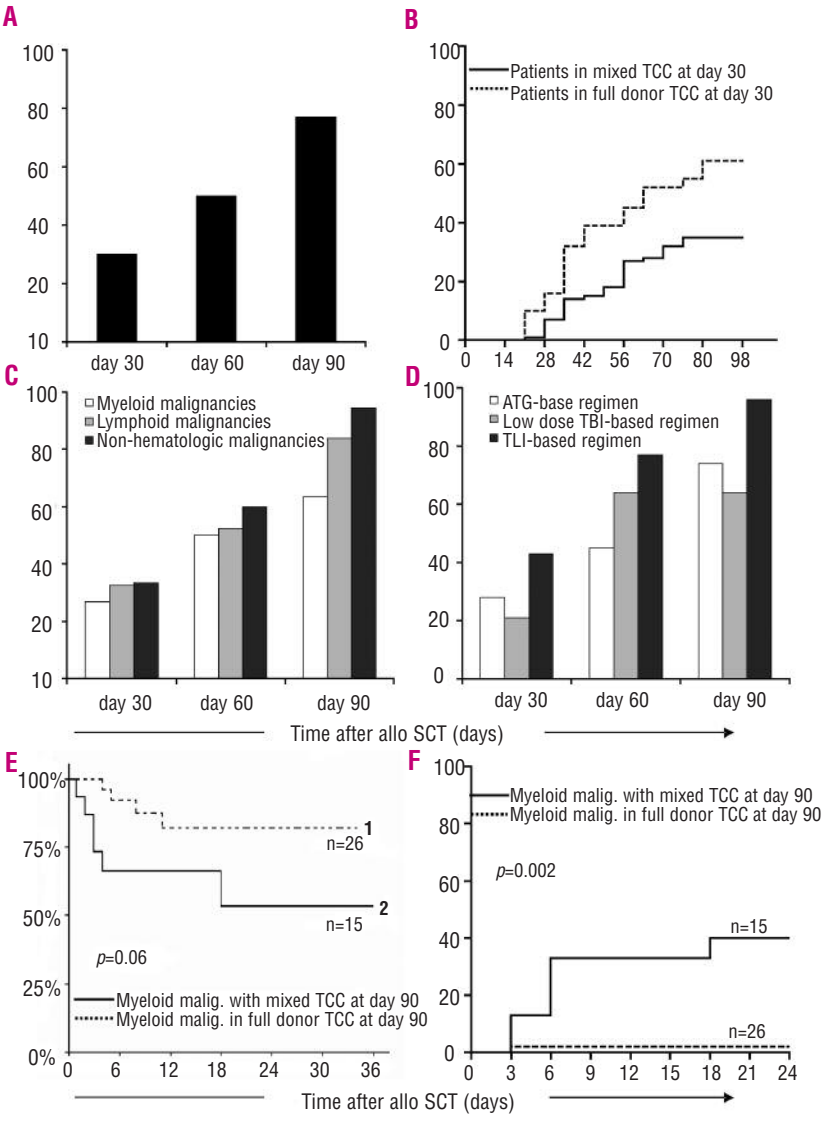

Figure 1. Kinetics of T-cell chimerism (TCC) after RIC allo-SCT. (A)% of patients achieving full donor TCC in the first three months after allo-SCT. Patients included in this study were assessed at all time points. (B) Cumulative incidence of grade 2-4 acute GVHD according to the TCC status at the end of the first month after allo-SCT. (C) Kinetics of TCC in the first three months after allo-SCT according to disease category. (D) Kinetics of TCC in the first three months after allo-SCT according to the RIC regimen type. (E) Progression-free survival in patients with myeloid malignancies according to TCC status at the end of the third month after allo-SCT. (F) Cumulative incidence of relapse in patients with myeloid malignancies (AML, CML, MDS) according to TCC status at the end of the third month after allo-SCT.

tivariate analysis, a diagnosis other than a myeloid malignancy, was predictive of full TCC at day 90 (p=0.007; OR=3.82;95%CI,1.4-10.1). The delayed full donor TCC in patients with myeloid malignancies (AML, CML, MDS) resulted in a poorer PFS (p=0.06; Figure 1E). This was also confirmed when analysis was restricted to the 24 patients with AML (p=0.017). Interestingly, there were no significant differences between disease risk factors in the AML patients' sub-group who achieved full donor TCC when compared to the other sub-group. This poorer PFS for myeloid malignancies was due to a higher incidence of relapse (6 relapses/15 patients in mixed TCC; 40%) compared to none in the 26 patients in full TCC (p=0.002; Figure 1F).

In this study, we identified factors influencing TCC following RIC-allo-SCT. The intensity of immunosuppression included in the RIC regimen is likely to influence establishment of TCC. The association between grade 2-4 acute GVHD and early full donor TCC, suggests that close monitoring of TCC may help clinical decision-making, as previously shown.[5,6] Disease category appears also to be a predictive factor for full donor TCC. This is delayed in patients with myeloid malignancies, which suggests that TCC is helped by prior exposure to high dose chemotherapy or to multiple lines of chemotherapy.[7] Therefore, patients less exposed to chemotherapy would require more intensive conditioning to achieve full donor TCC. This is extremely important because these patients (MDS, CML, AML) were found to have a higher incidence of relapse if they failed to achieve rapid full donor TCC. However, one must also consider the potential role of residual disease. Monitoring $CD34^+$ cells chimerism at day 30 may prove to be useful and is currently under investigation. In conclusion, monitoring of the kinetics of donor TCC is mandatory after RIC-allo-SCT, and can improve patients' outcome.

Mohamad Mohty,°,# Odile Avinens,@ Catherine Faucher,* Patrice Viens,^ Didier Blaise,*°,# Jean-Francois Eliaou@*

**Unite de Transplantation et de Therapie Cellulaire (UTTC), Institut Paoli-Calmettes, Marseille, France; °Université de la Méditerranée, Faculté de Médecine, Marseille, France; #INSERM, UMR 599, Marseille, France; @Laboratoire d'Immunologie, Hopital Saint-Eloi, CHU de Montpellier, Montpellier, France; ^Département d'Oncologie Médicale, Institut Paoli-Calmettes, Marseille, France*

DB and JFE share senior authorship.

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Correspondence: Mohamad Mohty, M.D., Ph.D., Institut Paoli-Calmettes, 232 Bd. Ste Marguerite, F-13273 Marseille Cedex 09, France. Phone: international +33 491 223823; Fax: international +33 491 223579. E-Mail: mohtym@marseille.fnclcc.fr

References

1. Mohty M, Bay JO, Faucher C, Choufi B, Bilger K, Tournilhac O, et al. Graft-versus-host disease following allogeneic transplantation from HLA-identical sibling with antithymocyte globulin-based reduced-intensity preparative regimen. Blood 2003;102:470-6.
2. Mohty M, Jacot W, Faucher C, Bay JO, Zandotti C, Collet L, et al. Infectious complications following allogeneic HLA-identical sibling transplantation with antithymocyte globulin-based reduced intensity preparative regimen. Leukemia 2003;17:2168-77.
3. Schichman SA, Suess P, Vertino AM, Gray PS. Comparison of short tandem repeat and variable number tandem repeat genetic markers for quantitative determination of allogeneic bone marrow transplant engraftment. Bone Marrow Transplant 2002;29:243-8.
4. Baron F, Baker JE, Storb R, Gooley TA, Sandmaier BM, Maris MB, et al. Kinetics of engraftment in patients with hematologic malignancies given allogeneic hematopoietic cell transplantation after nonmyeloablative conditioning. Blood 2004;104:2254-62.
5. Childs R, Clave E, Contentin N, Jayasekera D, Hensel N, Leitman S, et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. Blood 1999;94:3234-41.
6. Mattsson J, Uzunel M, Remberger M, Ringden O. T cell mixed chimerism is significantly correlated to a decreased risk of acute graft-versus-host disease after allogeneic stem cell transplantation. Transplantation 2001;71:433-9.
7. Carvallo C, Geller N, Kurlander R, Srinivasan R, Mena O, Igarashi T, et al. Prior chemotherapy and allograft CD34+ dose impact donor engraftment following nonmyeloablative allogeneic stem cell transplantation in patients with solid tumors. Blood 2004;103:1560-3.