


## ORIGINAL ARTICLE

# Utility of assessing CD3<sup>+</sup> cell chimerism within the first months after allogeneic hematopoietic stem-cell transplantation for acute myeloid leukemia

Mehdi Bendjelloul<sup>1,2</sup> | Cédric Usureau<sup>1,2</sup> | Pascaline Etancelin<sup>3</sup> |  
Zuzana Saidak<sup>4</sup> | Delphine Lebon<sup>2,5</sup> | Loïc Garçon<sup>1,2</sup> |  
Jean-Pierre Marolleau<sup>2,5</sup> | Judith Desoutter<sup>1</sup> | Nicolas Guillaume<sup>1,2</sup> 

<sup>1</sup>Department of Hematology and Histocompatibility, Amiens University Medical Center, Amiens, France

<sup>2</sup>EA HEMATIM 4666, Jules Verne University of Picardie, Amiens, France

<sup>3</sup>Department of Genetic Oncology, Centre Henri Becquerel, Rouen, France

<sup>4</sup>Laboratory of Molecular Oncobiology, Center of Human Biology, Amiens-Picardie University Medical Center, Amiens, France

<sup>5</sup>Department of Clinical Hematology and Cellular Therapy, Amiens University Medical Center, Amiens, France

## Correspondence

Nicolas Guillaume, Department of Histocompatibility, Amiens University Medical Center, F-80054 Amiens, France.  
Email: [guillaume.nicolas@chu-amiens.fr](mailto:guillaume.nicolas@chu-amiens.fr)

After allogeneic hematopoietic stem-cell transplantation (alloHSCT), the chimerism assay is used to monitor cell engraftment and quantify the respective proportions of donor/recipient cells in blood or bone-marrow samples. Here, we aimed to better assess the utility of determining CD3<sup>+</sup> cell chimerism within the first 6 months post alloHSCT. One hundred and thirty five patients diagnosed with acute myeloid leukemia were enrolled in this study. We observed significantly lower overall survival and relapse free survival for patients without full donor chimerism (<95%, <98%, <99%) in whole blood at Day 30, as well as at Day 90 after alloHSCT, than for patients with full donor chimerism. This outcome was not observed when assessing selected CD3<sup>+</sup> cells. However, at Day 90, patients with discordant whole blood versus selected CD3<sup>+</sup> cell chimerism showed both significantly lower overall survival and relapse free survival, giving an interest to assess selected cells chimerism.

## KEYWORDS

acute myeloid leukemia, chimerism, hematopoietic stem-cell transplantation, prognosis

## 1 | INTRODUCTION

Following allogeneic hematopoietic stem-cell transplantation (HSCT), the chimerism assay is the gold standard test used to monitor cell engraftment and quantify the respective proportions of donor/recipient cells in blood or bone-marrow samples. Chimerism assays are based on various genomic techniques, including short tandem-repeat (STR) assays, quantitative real-time polymerase chain reactions (qPCR), digital droplet PCR (ddPCR), and next-generation

sequencing (NGS).<sup>1</sup> All cell fractions are screened and, in addition, various lineage-specific cell subsets can also be usefully analyzed.<sup>2,3</sup> Consensus definitions from the American Society for Transplantation and Cellular Therapy have recommended that routine measurement of donor chimerism focus on CD3<sup>+</sup> cells, or similar, for lymphoid cells and CD133<sup>+</sup> cells, or similar, for myeloid cells.<sup>4</sup> Documentation of both myeloid and lymphoid engraftment is particularly relevant in the setting of non-myeloablative conditioning regimens.<sup>2</sup> Several studies have reported detectable lineage-specific mixed chimerism post-HSCT in association with clinical outcomes. Among them, one

Mehdi Bendjelloul and Cédric Usureau contributed equally to this work.

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showed CD3<sup>+</sup> sorted donor chimerism  $\leq 85\%$  at days +90 to +120 to be associated with a higher frequency of three-year disease progression in acute myeloid leukemia (AML).<sup>5</sup> In another study, performed on a pediatric population transplanted for malignant and non-malignant diseases, recipient chimerism levels in T cells  $< 50\%$  predicted a very low risk of rejection.<sup>6</sup> In monitoring for residual disease, cell sorting targeted to malignant clonal cells may increase the sensitivity of the detection of chimerism.<sup>7</sup> Moreover, monitoring

chimerism on CD34<sup>+</sup> cells appears to be more predictive of relapse in AML.<sup>8</sup> The utility of quantifying CD3<sup>+</sup> chimerism in selected cells to follow engraftment is controversial, particularly in cases of allograft after non-myeloablative or reduced intensity conditioning, in determining the risk of acute or chronic graft versus host disease (a- or cGVHD) and the risk of rejection.<sup>9–11</sup> In this context, we aimed to better assess the utility of determining CD3<sup>+</sup> cell chimerism within the first 6 months post alloHSCT.

**TABLE 1** Demographic characteristics of study population

		<b>OS</b>	<b>RFS</b>
		<b>p Values</b>	
Recipients ( <i>n</i> = 135)			
Age at transplantation, years, median (range)	55 (17–72)	0.32	0.32
Male	81 (60%)	0.23	0.32
Female	54 (40%)		
Recipient–donor serological status for CMV ( <i>n</i> = 139)			
Negative–Negative	59	0.41	0.41
Other combinations	77		
Recipient–donor gender ( <i>n</i> = 139)			
Male–Female	24	0.21	0.21
Other combinations	115		
Recipient–donor ABO status ( <i>n</i> = 129)			
ABO major mismatch	28	0.05	0.05
Others ABO compatibility	101		
Conditioning regimen ( <i>n</i> = 139)			
Reduced-intensity or non-myeloablative conditioning	113 (81%)	0.06	0.08
Myeloablative	26 (19%)		
Conditioning with ATG	6 (4%)	ND	
Disease stage at transplantation ( <i>n</i> = 139)			
Complete remission (CR1/CR2)	103 (81/24)	<0.005	<0.005
Non-complete	36		
Source of stem cells ( <i>n</i> = 139)			
Bone marrow	18 (13%)	0.03	0.03
Peripheral blood	121 (87%)		
Type of donor ( <i>n</i> = 139)			
HLA-fully-matched related	44 (32%)	>0.4	>0.4
HLA-haplo-matched related	28 (20%)		
HLA-fully-matched unrelated	62 (45%)		
HLA-fully-mismatched unrelated	5 (3%)		
Acute GVHD ( <i>n</i> = 125)			
Grade 0–I	90	0.71	0.62
Grades II–IV	35		
Follow up median months (range) ( <i>n</i> = 139)	25 (0–86)		

Abbreviations: ATG, anti-thymocyte globulin; CMV, cytomegalovirus; GVHD, graft versus host disease; ND, not done; OS, overall survival; RFS, relapse free survival.

TABLE 2 Link between CD34+, CFU-GM cell doses, clonogenic assay and whole blood chimerism at D30

Whole Blood chimerism at D30	>95%	<95%	p Values
CD34+ cell dose 10 <sup>6</sup> /kg of body weight infused (median-IQR)	6.13 ± 2.13	6.47 ± 1.97	0.42
CFU-GM cell dose 10 <sup>4</sup> /kg of body weight infused (median-IQR)	116.78 ± 73.88	127.40 ± 65.51	0.46
Clonogenic assay (%) (median-IQR)	18.68 ± 8.87	18.91 ± 7.57	0.89

Abbreviation: IQR, interquartile range.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

Consecutive patients diagnosed with AML ( $n = 135$ ) referred to the Hematology Clinic at the Amiens University Hospital, France, between June 2011 and January 2021 were enrolled in this study. In accordance with French legislation and the Declaration of Helsinki, all participants gave their prior, written, informed consent to genetic testing. Clinical data, such as overall survival (OS), relapse-free survival (RFS), and the demographic characteristics of the study population were retrospectively collected.

### 2.2 | qPCR chimerism monitoring

Ethylenediaminetetraacetic acid (EDTA) anticoagulated peripheral blood samples were obtained at days 30 (D30), 90 (D90), and 180 (D180) after alloHSCT. Lineage-specific separation was carried out within 4 h of venipuncture. CD3<sup>+</sup> cells were isolated from 7 ml peripheral blood samples using the EasySep<sup>®</sup> Human Whole Blood CD3 Positive Selection Kit (STEMCELL Technologies, Vancouver, BC, Canada). Genomic DNA was extracted from 350  $\mu$ l whole blood or selected CD3<sup>+</sup> cell subsets using an EZ1 Advanced XL Robotic Workstation and the EZ1 DNA Blood 350  $\mu$ l Kit (both from Qiagen, Hilden, Germany). Cell purity was assessed using the PCR-based Non-T Genomic Detection Kit (Accumol, Calgary, Canada) according to the manufacturer's instructions.<sup>12</sup> All samples included in this study showed purity better than 93% for the CD3<sup>+</sup> fraction. The QTRACE<sup>®</sup> assay (Jeta Molecular, Utrecht, Netherlands) was used to monitor qPCR chimerism, both on whole blood cells and CD3<sup>+</sup> lymphocytes.

### 2.3 | Statistical analysis

For statistical analysis, we defined full donor chimerism as >95% or >98% or >99%, mixed donor chimerism as

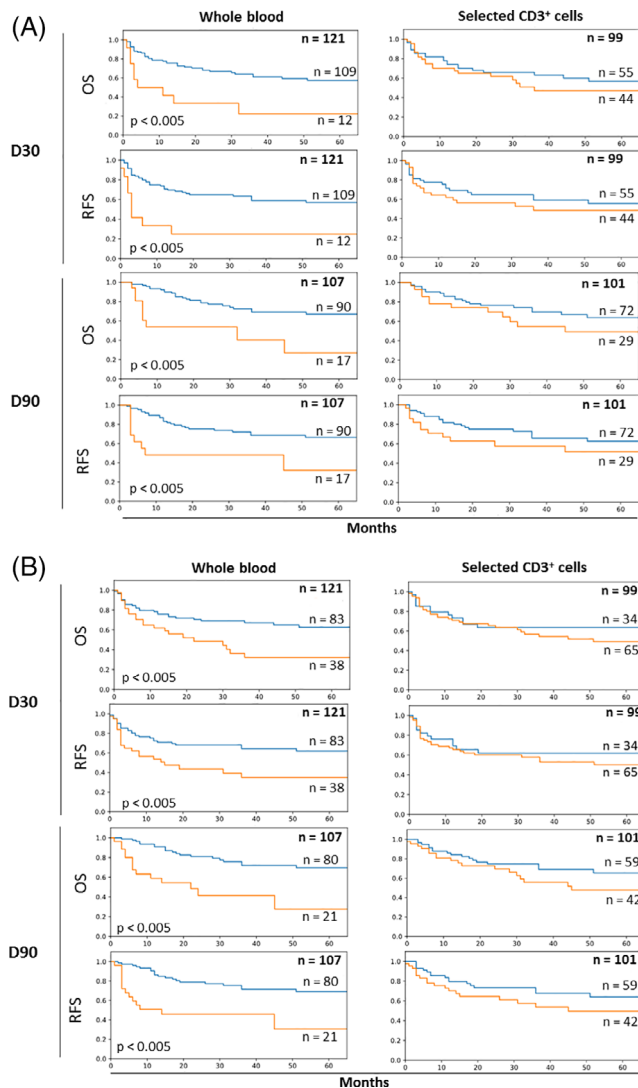
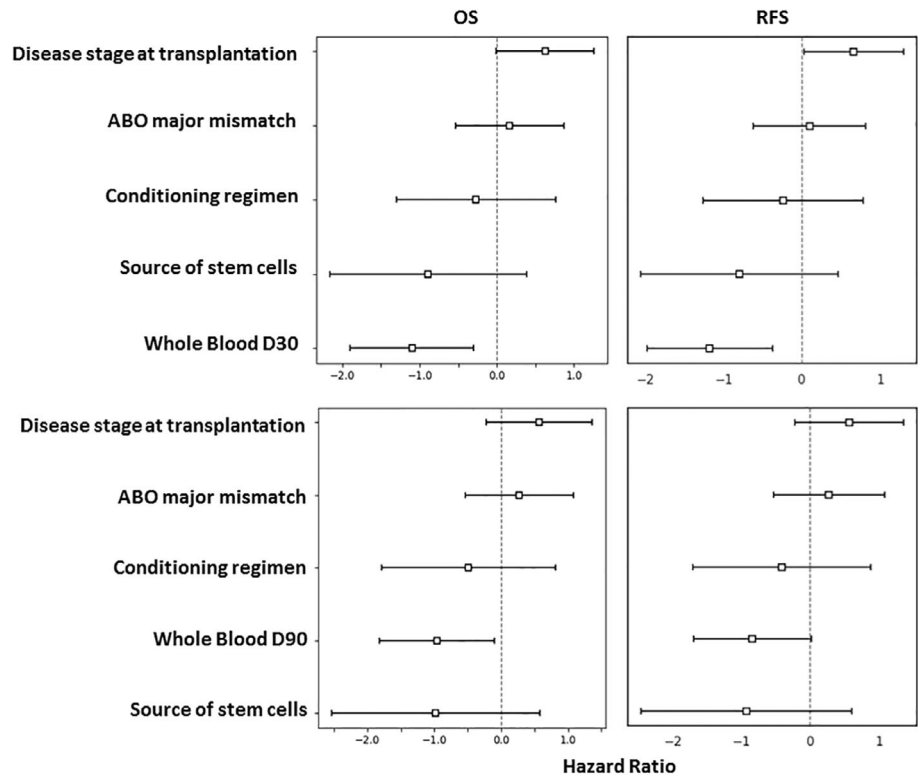


FIGURE 1 Impact of whole blood and selected CD3<sup>+</sup> cell chimerism on overall survival (OS) and relapse free survival (RFS) at day +30 (D30) and day +90 (D90). Graft survival analysis were death censored. Statistical analyzes about conditioning with anti-thymocyte globulin (ATG) have not been done ( $n = 6$ ). (A) Statistical analysis with a full donor chimerism >95%. Statistical analysis with a full donor chimerism >99%

5%–95% or 2%–98% or 1%–99%, and absent donor chimerism as <5% or <2% or <1%, both for whole blood cells and the lymphoid CD3<sup>+</sup> lineage. Kaplan–Meier type OS

**FIGURE 2** Impact of whole blood cell chimerism (full donor chimerism >95%) on overall survival (OS) and relapse free survival (RFS) at day +30 (D30) and day +90 (D90) according to the multivariate analysis by Cox regression



and RFS curves were plotted using R software. Differences in survival were estimated using the Mantel-Haenszel test (logrank) according to the chi-squared law. Differences with a  $p$  value <0.05 were considered statistically significant.

### 3 | RESULTS AND DISCUSSION

The clinical characteristics of all 135 included patients are presented in Table 1. In total, 139 allo-HSCTs were performed, including four patients who received a second allo-HSCT. Among the allo-HSCTs, 62 (45%) were performed with unrelated HLA-matched donors, 5 (3%) with unrelated HLA-mismatched donors, 44 (32%) with related HLA-fully-matched donors, and 28 (20%) with HLA-haplo-matched donors. One hundred and thirteen (81%) HSCTs were performed with reduced-intensity or nonmyeloablative conditioning and 26 (19%) with a myeloablative regimen.

The CD34<sup>+</sup> cell dose/kg of body weight infused after chemotherapy, known to be a major determinant of hematopoietic engraftment, was not in our study a determinant of chimerism in whole blood results at D30 regardless of the definition of full chimerism (full donor chimerism as >95% and also as >98 and >99%). This lack of link was also highlighted with the CFU-GM values and the clonogenic assays (Table 2 with full donor chimerism as >95%).

We observed significantly lower OS for patients without full donor chimerism (<95% or <98% or <99%) in whole blood at D30 ( $p < 0.005$ ), as well as at D90 ( $p < 0.005$ ), than for patients with full donor chimerism. This outcome was not observed when assessing selected CD3<sup>+</sup> cells, for which there was no significant difference in OS regardless of the percentage of donor chimerism at D30 or D90. Similarly, only whole blood chimerism had a significant impact on RFS, with lower RFS observed for patients without full donor chimerism (<95% or <98% or <99%) at D30 ( $p < 0.005$ ), as well as at D90 ( $p < 0.005$ ) (Figure 1A, full donor chimerism as >95%; Figure 1B, full donor chimerism as >99%). We also performed statistical analysis focusing only on the 113 patients who underwent the reduced-intensity or nonmyeloablative conditioning regimen, for whom we obtained the same results (data not shown).

Cox regression to investigate the effect of several variables with  $p < 0.1$ , such as disease stage at transplantation, ABO major mismatch, conditioning regimen, and source of stem cells, still showed statistical significance for the effect of chimerism in whole blood cells for OS and RFS at D30 ( $p < 0.005$ ) and D90 ( $p < 0.005$ ) and for the disease stage at transplantation regardless of the definition of full chimerism (full donor chimerism as >95% and also as >99%) (Figure 2).

However, patients with discordant whole blood versus selected CD3<sup>+</sup> cell chimerism showed both significantly lower OS ( $p = 0.007$ ) and RFS ( $p = 0.011$ ) at D90 only.

There were 22 “discordant” patients with at least one of the two chimerism values <95% (of which 17 patients with CD3<sup>+</sup> cell chimerism <95% versus 66 whose chimerism was concordant and >95% on whole blood cells and selected CD3<sup>+</sup> cells. With a full donor chimerism >99%, we have individualized 15 discordant patients (of which 13 patients with CD3<sup>+</sup> cell chimerism <99% versus 73 whose chimerism was concordant with a value >99%). On these 15 discordant patients, only 8 patients showed full donor chimerism at D180, 7 patients were in relapse or deceased. By D180, most of the patients in our cohort showed full donor chimerism and statistical analysis could not be performed.

In terms of clinical data, using the chi-squared distribution, we did not show a link between the occurrence of aGVH grade II-IV and whole blood cells and donor CD3<sup>+</sup> selected cell chimerism >95%, >98%, and >99%. Others parameters such as recipient-donor gender, CMV status, ABO status, disease stage at transplantation, source of stem cells, conditioning regimen and type of donor failed also to show a link with aGVH occurrence.

Finally, our chimerism assay showed a weak correlation with the minimal residual disease status (MRD). Indeed, at D30, on the 16 patients with a well-defined positive MRD, only 9 have a whole blood chimerism <99%. At D90, on the 11 patients with a positive MRD, only 5 have a whole blood chimerism <99%.

Thus, the added clinical value of monitoring selected CD3<sup>+</sup> cell chimerism at D30 appears to be limited, in addition to the failure encountered in extracting sufficient genomic DNA from the minority CD3<sup>+</sup> cell lines a few days after recovery from aplasia. This disparity between the two results may be due to contamination attributable to residual recipient lymphocytes, notably in non-myeloablative regimens. However, at D90, the discrepancy between the chimerism observed in whole blood and that in selected CD3<sup>+</sup> cells provides useful information for patient monitoring due to its association with a significant decrease in survival. Furthermore, for these patients, therapeutic actions, such as stopping immunosuppression or proceeding with donor lymphocyte infusion can be addressed.

## CONFLICT OF INTEREST

The authors declare no competing financial interests.

## AUTHOR CONTRIBUTIONS


Mehdi Bendjelloul, Judith Desoutter, and Nicolas Guillaume designed and performed the experiments, analyzed and interpreted the data, and wrote the paper; Cédric Usureau and Zuzana Saidak performed the

statistical analysis; Pascaline Etancelin, Delphine Lebon, Loïc Garçon, and Jean-Pierre Marolleau provided clinical data and reviewed the manuscript for critical content.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

Nicolas Guillaume  <https://orcid.org/0000-0003-2573-5989>

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