Efficacy of minimal residual disease driven immune-intervention after allogeneic hematopoietic stem cell transplantation for high-risk chronic lymphocytic leukemia: results of a prospective multicenter trial

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Supplemental method

Study design

The ICLL03 RICAC-PMM (Reduced Intensity Conditioning Allogeneic Transplantation for CLL with Preemptive MDR Management) was a multicenter phase II trial, on behalf of the FILO (French Innovative Leukemia Organization) and the SFGM-TC (Société Francophone de Greffe de Moelle et de Thérapie Cellulaire) groups.

The 2016 EBMT consensus applied for allogeneic hematopoietic stem cell transplantation (HSCT) included patients with one or more of the following features : (i) refractoriness or early relapse (within 12 months) after treatment with a purine analogue; (ii) relapse within 24 months after treatment association including a purine analogue or after an autologous stem cell transplantation (SCT); or (iii) presence of del(17p) deletion and/or TP53 mutations with treatment indication. (Dreger, Leukemia, 2007)

Exclusion criteria included Richter's transformation, serious non-controlled infections, concomitant malignant disease, impaired organ function, HIV seropositivity and psychiatric disease.

Response and MRD evaluation

For response evaluation, formal assessment of clinical status, CT-scan and full blood count were scheduled at 3 months (M3), 6 months (M6) and 12 months (M12) post transplantation. Bone marrow biopsy was planned to confirm CR or CRi, if not already confirmed at screening.

MRD analysis was centrally performed in the Hematology Laboratory of the Pitié-Salpêtrière hospital in Paris on blood and/or bone marrow by 10-color multiparameter flow cytometry according to the international harmonized recommendation (Rawstron et al., 2007, 2013, 2016) to which the isotypic restriction evaluation (kappa and lambda light chains) was added.

The single-tube assay includes 11 antibodies, namely: Kappa-FITC (Dako, Les Ulis, France)/ Lambda-PE (Dako) / CD3+CD14 ECD (Beckman Coulter, Villepinte, France) / CD19 PC5.5 (Beckman Coulter)/ CD5 PC7 (BD Biosciences, Le Pont de Claix, France)/ CD81 APC (Pharmingen, Le Pont de Claix, France)/ CD22 APC AF700 (Beckman Coulter)/ CD43 APC AF750 (Beckman Coulter)/ CD20 Pacific Blue (Beckman Coulter)/ CD45 Krome orange (Beckman Coulter). A sufficient volume of blood was lysed with the VersaLyse reagent (Beckman Coulter) in order to obtain enough leucocytes to reach a limit of detection of 1 CLL cell in 1,000,000 leukocytes (i.e 0.0001% or 10^{-6}). The median limit of detection (LOD) achieved was 4.10^{-6}.

Navios flow cytometer (Beckman Coulter) was used for the acquisition of cells and Kaluza software (Beckman Coulter) was used for data analysis. Clusters of 20 events or more were considered as evidence for MRD. MRD levels were calculated by dividing the number of CLL cells by the number of leukocytes. MRD_{neg} status was defined when <1 CLL cell was detectable per 10,000 leukocytes.[19] A positive MRD (MRD_{pos}) was defined when ≥ 1 positive cell was detectable per 10,000 leukocytes. Clusters of less than 20 events were considered as undetectable MRD (UD). For each UD evaluation point, the limit of detection (LOD) is calculated as the ratio of 20 by the number of total analyzed leukocytes.

Preemptive immune-intervention

Preemptive immune-intervention was based on the response and blood MRD assessment and was applied in absence of significant GVHD, defined by either acute GVHD (aGVHD) ≥ grade II or extensive chronic GVHD (cGVHD). The algorithm (Supplemental Figure A), included acceleration of CsA tapering and withdrawal

followed in case of failure by escalating DLI was applied according to both response and MRD evaluation. The algorithm also included extension of CsA treatment in case of early achievement of negative MRD status. At day 30, in patients with progressive disease (PD), CsA was tapered over 12 days. At day 60 (D60), in patients who did not achieve CR/CRi, CsA was tapered over 28 days. At day 90 (D90), in patients with remaining MRDpos status, CsA was tapered over 56 days. Finally, patients not responsive 4 weeks after CsA withdrawal and who had no significant GVHD, received escalating doses of 1 to 3 DLI. The starting DLI dose was 5x106 CD3/kg of recipient in PD at day 30 and not in CR/CRi at D60 and 1x106 CD3/kg in MRDpos patients. In absence of significant GVHD, subsequent increased doses of DLI were infused every 8 weeks. Conversely, for patients in CR/CRi and with early blood MRDneg status, CsA decrease was delayed, starting at day 120 for D90 MRDneg patients and at day 150 for D60 MRDneg patients. CsA was stopped after 2 months tapering in both cases.

Chimerism evaluation

Chimerism was measured (after comparison of donor and recipients profiles) by multiplex fluorescent PCR using Short Tandem Repeat analysis (Identifiler+ Kit on ABI 3500 Sequencer, Life Technologies, Villebon/Yvette, France) on DNA from total peripheral blood or CD3 T cells (isolated with the positive selection MidiMACS technology using Whole Blood CD3 Microbeads and Whole Blood Column kit, Myltenyi Biotec, Paris, France). The sensitivity of the method is 1%. Total donor chimerism was defined as ≥ 95% donor chimerism, mixed chimerism as donor chimerism between 5 and 95%, and graft failure as donor chimerism < 5%. As per protocol, the chimerism results were not a part of the algorithm of preemptive immune-intervention.

Trial objectives and statistical analysis

The sample size estimation was estimated according to one-stage Fleming design for a two-sided type I error at 5% and statistical power equals 95%, with thresholds of MRDneg status at 45% and 70%. This estimation was then considered as acceptable considering a planned 3 years accrual and the incidence of allogenetic HSCT in CLL in France.

Type 1 error was fixed at the 5% level. All tests were 2-tailed. A final point of follow-up and analysis was performed in April 2017. All analyses were performed using Stata software (version 13, StataCorp, College Station, TX).

Supplemental tables

Supplemental table #1: Lines of treatment prior to HSCT

Pt#	Line #1	Line #2	Line #3	Line #4	Line #5
1	AD	AD			
2	AD				
3	mCHOP+F	FCR	FCR+R	BR	R-DHAC
4	CVP	R-CHOP	FCR	BR	
5	AD	A			
6	FCR	BR			
7	AD				
8	FCR	BR			
9	BR	R-DHAC			
10	AD				
11	A				
12	FCR	BOMP			
13	BR	R-DHAC			
14	FCR	FC	B		
15	A				
16	FCR	BR	R-DHAC		
17	FCR+BEAM/auto	FCR	BR		
18	AD				
19	FCR	BOMP			
20	AD				
21	A	BOMP			
22	FCR	FCR	BOMP		
23	AD				
24	R+R	BR	R		
25	FCR+R	AD			
26	A				
27	FCR	R-DHAC			
28	FC	ClbR	A		
29	FCR	BR	A		
30	FCR	BR	R-DHAC		
31	FCR	BR			
32	FCR	Ofa	BR		
33	AD	Ibr			
34	AD				
35	FC	BR	R-DHAC	R-CHOP	BR
36	FC	Clb	FCR	BOMP	
37	FCR	BR	AD	Ofa	Ibr
38	CHOP	FCR	A		
39	FCR	R-DHAP/C	BR	Ibr	
40	A				
41	FCR	AD	BOMP	Ibr	IdeR
42	FCR	R-mCHOP	BR		

Abbreviations: Pt# : patient number ; A : alemtuzumab ; AD : alemtuzumab and dexamethasone ; mCHOP+F : cyclophosphamide, doxorubicin, vincristine, prednisone (attenuated doses) plus fludarabine ; FCR : fludarabine cyclophosphamide and rituximab ; FCR : fludarabine cyclophosphamide and rituximab plus rituximab maintenance. BOMP : bendamustine, ofatumumab, methylprednisolone ; BR : bendamustine, rituximab ; Clb : chlorambucil ; ClbR : chlorambucil, rituximab ; CVP : cyclophosphamide, vincristine, prednisone ; Ibr : ibrutinib ; Ide-R : idelalisib, rituximab ; Ofa : ofatumumab ; R : Rituximab ; R-CHOP and R-mCHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone ; R-DHAC : rituximab, dexamethasone, cytarabine, carboplatinum.

Supplemental table #2: Correlation between the clinical evaluation (according to IWCLL criteria) at 3-6 months and the MRD status at 6 and 12 months.

	Evaluation at 3-6 months					
	CR	**PR**				**NE**
		LN	LN and S	S	ID	
	13	6	3	4	13	3
MRD 6 months						
Positive	2	3	2	1	4	0
Negative	11	3	1	3	9	0
NE	0	0	0	0	0	3
MRD 12 months						
Positive	0	2	3	1	1	0
Negative	11	4	0	3	8	0
NE	2	0	0	0	4	3

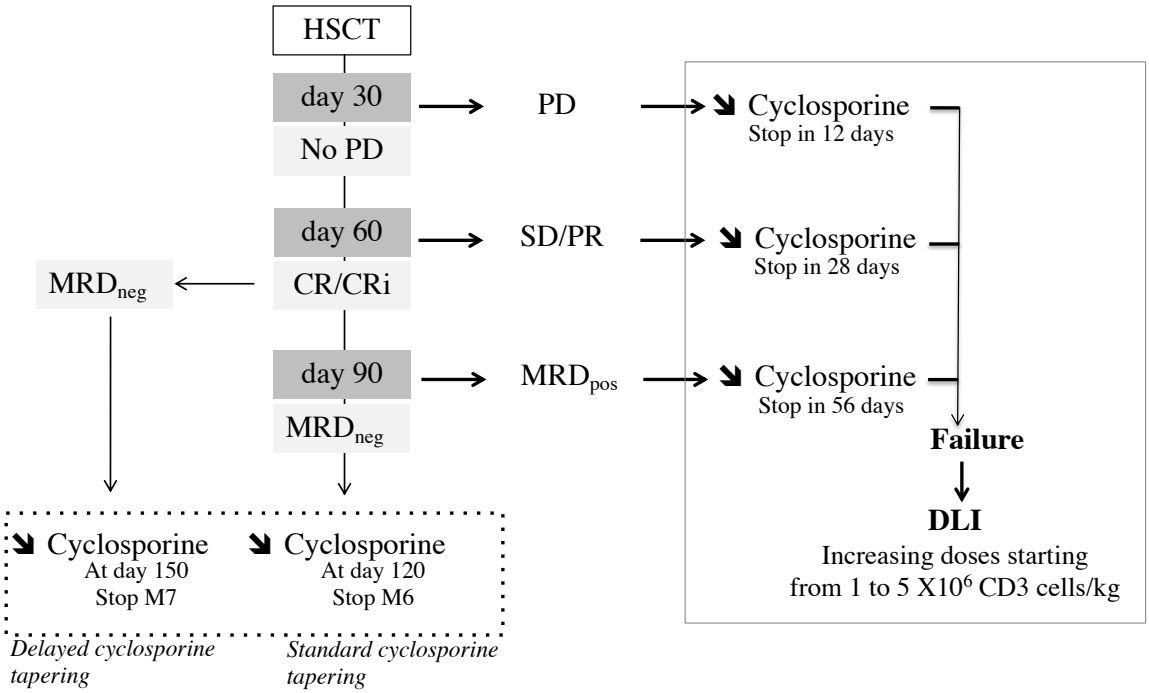
LN : persistent of lymph node(s) > 15 mm, S : persistence of spleen enlargement, ID : insufficient data to confirm a CR (CT-scan and/or Bone marrow trephine biopsy lacking).

Supplemental table #3: Patients follow-up according to MRD patterns.

Patterns	3y OS (%)	3y PFS (%)	Rel. (n)	TTR (m)	Death Cause (n)	TTD (m)	CsAi (m)	TTCsAi (d)	aGVH (n)	cGVH (n)	cGVH onset (d)
A (n=6)	100	66.7	2	12 ; 19	/		6	182 (164-231)	0	Limited (3)	118 (90-307)
B (n=11)	81.8	72.7	1	12	NRM (2)	10 ; 12	5	239 (144-279)	8	Limited (6) Extensive (2)	162 (90-409)
C (n=15)	100	82.5	2	13 ; 34	/	/	12	115 (27-265)	6	Limited (4) Extensive (6)	124 (90-167)
D (n=7)	68.6	14.3	5	1 ; 9 ; 11 ; 12 ; 12	NRM (1) Rel. (3)	9 35 ; 43 ; 47	6	134 (109-145)	3	Limited (2) Extensive (1)	95 (90-231)

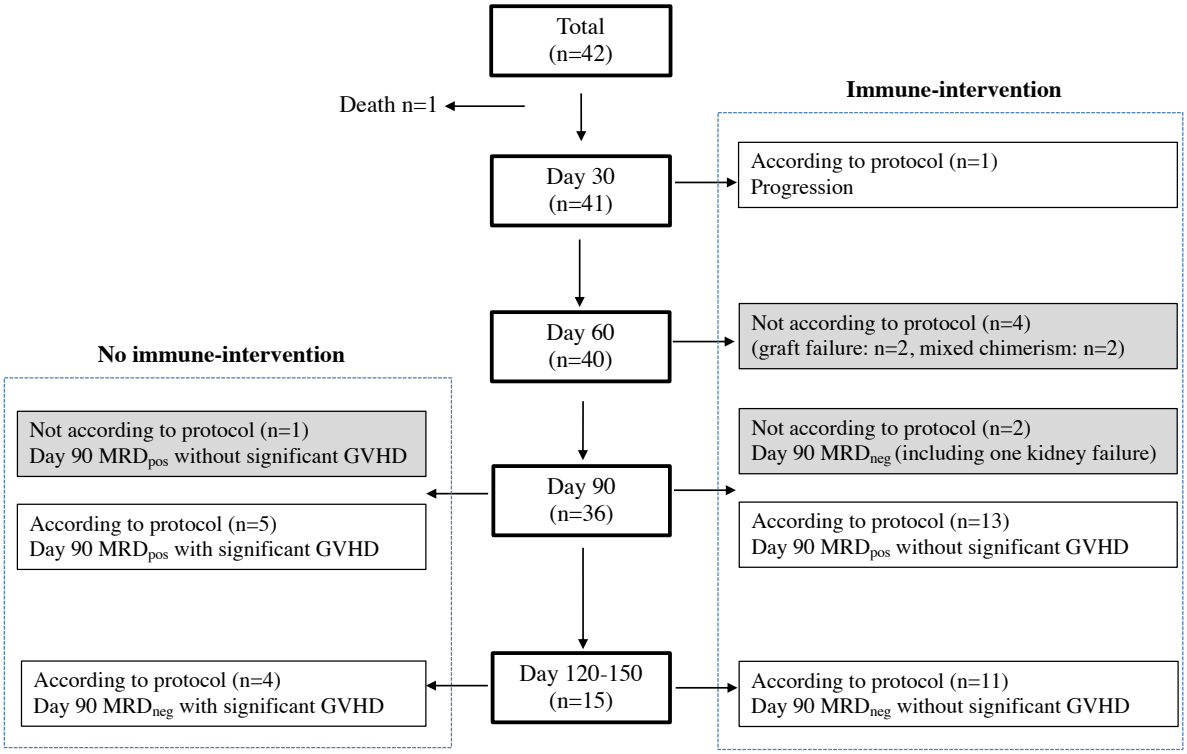
OS : overall survival, PFS : progression free survival, Rel. : relapse, TTR : time to relapse, TTD : time to death ; NRM : non relapse mortality, CsAi : Ciclosporine interruption within 12 months ; TTCsAi : time to cyclosporine interruption when recorded within 12 months, m : months, d : days, n : number.
For TTCsAi and cGHV onset, the median result is given with the extremes in brackets.

Supplemental figures

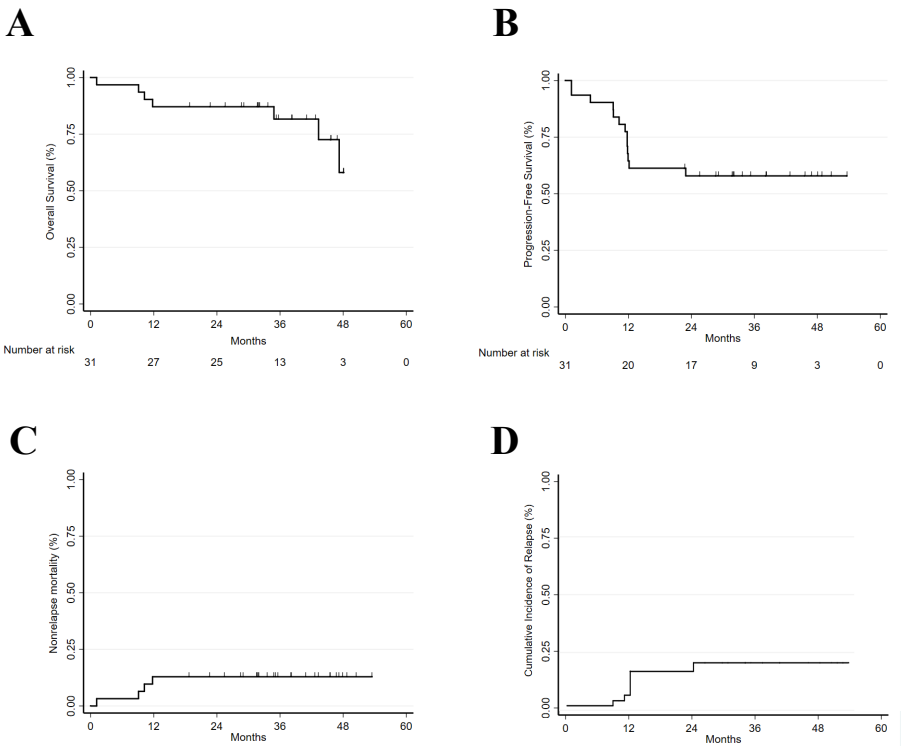


Supplemental Figure A: Algorithm of preemptive immune-intervention. The algorithm based on both response and MRD assessment was applied in the absence significant GVHD (aGVHD ≥ II and/or extensive cGVHD). For persistent detectable disease, the algorithm included acceleration of CsA tapering and withdrawal followed in case of failure by escalating DLI. The algorithm also included extension of CsA treatment in case of early achievement of negative MRD status.

HSCT: Hematopoietic stem cell transplant. PD: progressive disease. PR: partial response. SD: stable disease. CR: complete response. CRi: CR with incomplete marrow recovery. MRD_{neg}: negative minimal residual disease. $MRD_{pos:}$ positive minimal residual disease GVHD: graft versus host disease. DLI: donor lymphocyte infusion.



Supplemental Figure B: Protocol adherence according to trial profile of immune-intervention. Significant GVHD included grade II-IV acute GVHD or extensive chronic GVHD. One patient died early after transplantation and was not evaluated for protocol adherence.



Supplemental Figure C: Post-transplant outcome of the 39 CLL transplanted patients excluding patients treated in first line. Kaplan-Meier estimates of (A) overall survival, (B) progression free survival. Calculated probability of (C) non relapse mortality and (D) cumulative incidence of relapse after HSCT.