13th International Conference on Malignant Lymphoma, Lugano, Switzerland, June 17-20, 2015

Response to rituximab in B-Chronic Lymphocytic Leukemia (B-CLL) patients is adversely impacted by frequency of IL-10 competent B-CLL cells and FCGR3A polymorphism.

CLL2010-FMP (for fit medically patients): A study of the French Cooperative Group on CLL and Waldenström macroglobulinemia (WM) (FCGCLL/MW) and the "Groupe Ouest-Est d'études des Leucémies Aigües et autres Maladies du Sang" (GOELAMS).

A-L Gagez[1,2], E Tuaillon[3,4], R Cezar[4], C Cartigeas[5], B Mahé[6], R Letestu[7], H Maisonneuve[8], V Gouilleux-Gruat[2,9], K Bollore[3], E Ferrant[10], T Aurran[11], P Feugier[12], S Leprêtre[13], G Cartron[1,2,14]

[1]CNRS UMR-5235, Montpellier; [2]LabEx MAbImprove; [3]INSERM U1058, Montpellier; [4]CHRU Montpellier; [5]CHRU Tours; [6]CHRU Nantes; [7]CHU Bobigny; [8]CHD La Roche sur Yon; [9]CNRS UMR-7292, Tours; [10]CHRU Dijon; [11]Centre Paoli Calmette, Marseille; [12]CHRU Nancy; [13]Centre Henri Becquerel, Rouen; [14]CHRU Montpellier.

BACKGROUND

Rituximab has dramatically improved outcome of CLL.

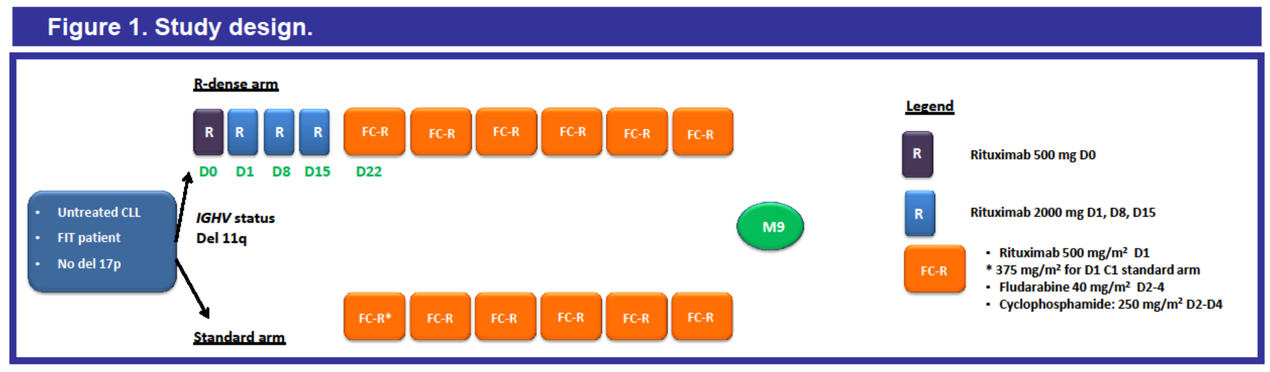
Its *in vivo* mechanisms of action remain unknown but some factors influencing response to rituximab have been described such polymorphism of the low affinity immunoglobulin gamma Fc region receptor III-A (CD16a, FCGR3A)[1].

In murine model, regulatory B-cells (B10) have been described to inhibit lymphoma cells clearance induced by anti-CD20 monoclonal antibodies through IL-10 negative regulation of monocyte Fc-mediated functions.[2-4] A sub-population of CLL B-cells has capacity to secrete IL-10 and could therefore influence rituximab activity in CLL patients.[5]

The goal of this work was to evaluate the role of IL10 competent B-CLL cells and *FCGR3A* polymorphism on *in vivo* rituximab activity in CLL patients receiving a pre-phase of high-dose of rituximab before conventional immune-chemotherapy.

PATIENTS AND TREATMENT

Figure 1. Study design.



METHODS

IL-10 competent CLL cells counts were determined by flow cytometric analysis on purified peripheral blood mononuclear cells after polyclonal stimulation.
Plasma concentration of Il-10 was measured by ELISA.
FcγRIIIA-158VF polymorphism was determined by PCR.
Rituximab pre-phase efficacy was defined by lymphodepletion >90% at D22, before the beginning of immuno-chemotherapy.
Response to treatment was evaluated 3 months after the end of immuno-chemotherapy according to IWCLL 2008 international criteria.

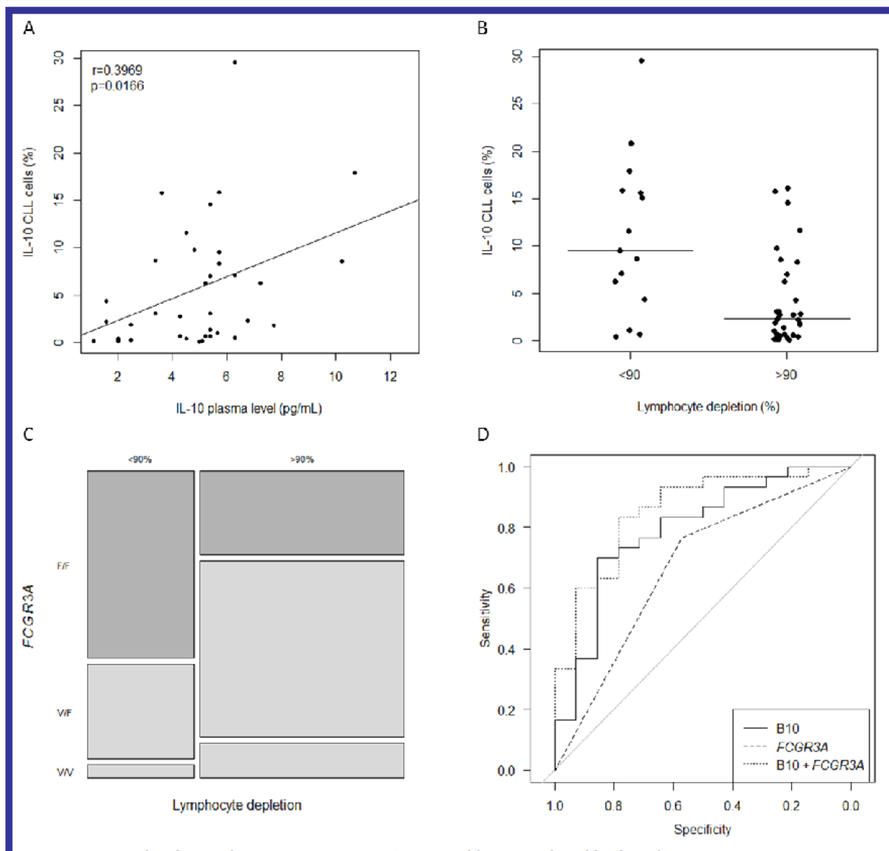
RESULTS

Table 1. Patients characteristics and parameters influencing lymphocyte depletion induced by rituximab monotherapy.

	Lymphodepletion >90% (n=44)		Lymphodepletion ≤90% (n=23)		Univariate analysis			Multivariate analysis	
	N (%)	Median (IQR)	N (%)	Median (IQR)	OR [95% IC]	p-value	AUC [95% CI]	OR [95% CI]	p-value
Age (years)	44 (100.00)	55.72 (51.31-58.12)	23 (100.00)	53.99 (52.07-57.41)	-	0.792	-		
Men	31 (70.45)	-	18 (78.26)	-	0.68 [0.19-2.16]	0.693	-	-	-
Binet stage AB	36 (81.82)	-	16 (69.57)	-	0.51 [0.15-1.73]	0.404	-	-	-
ECOG 0	31 (70.45)	-	5 (21.74)	-	1.50 [0.41-6.29]	0.572	-	-	-
IGHV unmutated	25 (56.82)	-	16 (72.73)	-	0.50 [0.15-1.50]	0.324	-	-	-
Cytogenetic abnormalities									
Del(13q)	18 (51.43)	-	8 (44.44)	-	1.31 [0.41-4.29]	0.848	-	-	-
Del(11q)	7 (16.67)	-	6 (26.09)	-	0.57 [0.16-2.07]	0.560	-	-	-
Trisomy 12	2 (6.06)	-	2 (14.29)	-	0.40 [0.03-6.04]	0.572	-	-	-
β2 microglobulin (mg/L)	39 (88.64)	2.90 (2.33-3.66)	22 (95.65)	2.76 (2.33-4.29)	-	0.857	-	-	-
CD38+ (%)	34 (77.27)	2.00 (0.00-23.00)	17 (73,91)	10.50 (1.75-26.00)	-	0.133		-	-
IL-10 competent cells (%)	32 (72.73)	2.30 (0.68-6.47)	15 (65.22)	9.51 (5.35-15.70)	-	0.004	0.763 [0.604-0.921]	0.83 [0.72-0.93]	0.002
FCGR3A					-	0.028	-	-	-
V/V	5 (11.91)	-	1 (4.55)	-					
V/F	25 (59.52)	-	7 (31.82)	-					
F/F	12 (28.57)	-	14 (63.63)	-					
FCGR3A V carrier	30 (71.43)	-	8 (36.36)	-	4.23 [1.43-13.42]	0.014	0.675 [0.551-0.799]	4.95 [1.07-27.48]	0.043

- No significant correlation was found between 90% lymphodepletion and clinical parameters: age, sex, Binet stage, ECOG, IGHV mutation, cytogenetic abnormalities (del11q, del13q, trisomy 12) or β2- microglobulin.
- No significant correlation was found between IL-10 cells and clinical parameters.

Figure 2. Effects of IL-10 CLL cells frequency and *FCGR3A* polymorphism on lymphocyte depletion induced by rituximab in CLL patients.



(A) IL-10 CLL cells frequency correlated statistically with IL-10 plasma level.
(B) Correlation between IL-10 cells and 90% lymphodepletion (p=0.004).
(C) FcγRIIIa-158V/F polymorphism was associated with 90% lymphodepletion (p=0.014).
(D) ROC curve: IL-10 cell frequency and FcγRIIIa-158VF were the only prognostic factors associated with lymphocyte depletion induced by rituximab alone.

- Correlation between IL-10 cells and clinical response assessed at M9: CR vs no-CR (p=0.04).
- FcγRIIIa-158V/F polymorphism failed to correlate with clinical response at M9: CR vs no-CR.

CONCLUSIONS

All these data strongly suggest that IL-10 competent B-CLL cells negatively regulate rituximab-mediated antibody-dependent cellular cytotoxicity by macrophages, this effect being also influenced by *FCGR3A* polymorphism.
Strategies targeting IL-10 mediated inhibitory effects should be consider to improve rituximab efficacy.

References

[1]Cartron G, et al. Blood. 2002;99(3):754-758.
[2]Matsushita T, et al. J Clin Invest. 2008;118(10):3420-3430.
[3]Yanaba K, et al. Immunity. 2008;28(5):639-650.
[4]Iwata Y, et al. Blood. 2011;117(2):530-541.
[5]DiLillo DJ, et al. Leukemia. 2013;27(1):170-182.

Acknowledgements

This work is supported by a public grant overseen by the French National Research Agency (ANR) as part of the "Investissements d'Avenir" program (reference: ANR-10-LABX-53-01).