Chemotherapy-induced augmentation of T cells expressing inhibitory receptors is reversed by treatment with lenalidomide in chronic lymphocytic leukemia

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Supplementary Table 1. Donor characteristics

Parameters	No. Donors (%)
Healthy controls:	
Total number	**14 (100)**
Sex	
Male	5 (36)
Female	9 (64)
Age (years)	
Mean	66.1
Range	45-88
Chemonaive CLL patients:	
Total number	**82 (100)**
Sex (nd: 1)	
Male	45 (56)
Female	36 (44)
Age (years)	
Mean	69.6
Range	46-88
Duration of disease (years)	
Mean	6.2
Range	0.2-16.4
Leukocytes (G/L)	
Mean	40.8
Range	3.2-140.4
Modified RAI stage (nd: 10)	
Low (0)	55 (76)
Intermediate and High (II-IV)	17 (24)
Molecular risk parameters	
Unmutated IgVH (nd: 22)	10 (17)
Unfavorable genetic aberration(s) (del17p, del11q, tri12) (nd: 20)	8 (13)
CD38+ CLL cells (≥ 30%) (nd: 1)	21 (26)
High Zap70 expression (nd: 6)	20 (26)

non-Lena treated CLL patients:

Total number	**39 (100)**
Sex	
Male	28 (72)
Female	11 (28)
Age (years)	
Mean	72.1
Range	53-90
Duration of disease (years)	
Mean	9.2
Range	1.0-31.2
Leukocytes (G/L)	
Mean	18.3
Range	1.5-171.0
Modified RAI stage (nd: 3)	
Low (0)	5 (14)
Intermediate and High (II-IV)	31 (86)
Molecular risk parameters	
Unmutated IgVH (nd: 12)	12 (44)
Unfavorable genetic aberration(s) (del17p, del11q, tri12) (nd: 9)	15 (50)
CD38+ CLL cells (≥ 30%) (nd: 1)	21 (55)
High Zap70 expression (nd: 7)	12 (38)

Lena treated CLL patients:

Total number	**10 (100)**
Sex	
Male	5 (50)
Female	5 (50)
Age (years)	
Mean	72.7
Range	60-84
Duration of disease (years)	
Mean	8.9
Range	4.8-14.3
Leukocytes (G/L)	
Mean	8.6
Range	2.2-20.8
Modified RAI stage (nd: 1)	
Low (0)	0 (0)
Intermediate and High (II-IV)	9 (100)
Molecular risk parameters	
Unmutated IgVH (nd: 5)	2 (40)
Unfavorable genetic aberration(s) (del17p, del11q, tri12) (nd: 3)	2 (29)
CD38+ CLL cells (≥ 30%) (nd: 1)	5 (56)
High Zap70 expression	6 (60)

Ig, immunoglobulin; VH, variable heavy chain; nd, not determined

Supplementary Table 2. Therapy details of treated patients in this study

Patient ID	time from last treatment (months)	last treatment regime
61	**16**	**AGMT CLL-5**
200	**41**	**AGMT CLL-5**
283	**30**	**AGMT CLL-5**
298	**38**	**AGMT CLL-5**
414	**50**	**AGMT CLL-5**
434	**48**	**AGMT CLL-5**
460	**43**	**AGMT CLL-5**
528	**12**	**AGMT CLL-5**
321	**12**	**Alemtuzumab**
361	**46**	**Alemtuzumab**
467	**14**	**Alemtuzumab**
337	**0**	**Bendamustin**
8	**15**	**Bendamustin/Alemtuzumab**
7	**33**	**Bendamustin/Rituximab**
181	**2**	**Bendamustin/Rituximab**
204	**3**	**Bendamustin/Rituximab**
234	**7**	**Bendamustin/Rituximab**
538	**38**	**Bendamustin/Rituximab**
590	**6**	**Bendamustin/Rituximab**
227	**10**	**Bortezumib/Dexamethasone/Rituximab**
75	**229**	**COP**
60	**111**	**FCR**
67	**56**	**FCR**
76	**46**	**FCR**
117	**90**	**FCR**
149	**20**	**FCR**
165	**80**	**FCR**
228	**52**	**FCR**
268	**56**	**FCR**
299	**41**	**FCR**
300	**20**	**FCR**
305	**51**	**FCR**
308	**39**	**FCR**
309	**53**	**FCR**
358	**72**	**FCR**
366	**17**	**FCR**
435	**11**	**FCR**
523	**30**	**FCR**
557	**5**	**FCR**
2	**93**	**Fludarabine**
45	**112**	**Fludarabine**
70	**45**	**Fludarabine**
111	**114**	**Fludarabine/Rituximab**
155	**68**	**Fludarabine/Rituximab**
187	**59**	**GUPTA**
304	**68**	**GUPTA**
287	**53**	**Lenalidomide**
240	**45**	**Lenalidomide/FCR**
427	**19**	**Rituximab**

The median interval between last therapy and cell isolation was 45 months (0-229).

GUPTA:	Prednisolone+Cyclophosphamide+Rituximab
FCR:	Fludarabine+Cyclophosphamide+Rituximab
COP:	Vincristine+Cyclophosphamide+Prednisone
AGMT CLL-5:	Fludarabine+Rituximab+Lenalidomide

Supplementary Table 3: Significance levels (p-values) of correlation of exhaustion marker expression with clinical risk parameters in 82 chemonaive CLL patients

		CD38 low vs. high		zap70 low vs. high		IgVH mutated vs. unmutated		favourable vs unfavourable cytogenetic		RAI 0 vs RAI 1-4		leukocyte counts	
CD4+	PD-1+	40.7 vs 46.3 %	p=.069	43.0 vs 41.3 %	p=.683	41.0 vs 47.9 %	p=.219	39.0 vs 48.2 %	p=.092	**39.4 vs 48.6 %**	**p=.033**	r=.100	p=.407
	2B4+	13.2 vs 12.6 %	p=.718	15.1 vs 8.2 %	p=.229	14.0 vs 16.7 %	p=.599	10.4 vs 15.1 %	p=.419	12.3 vs. 15.7%	p=.533	r=-.121	p=.314
	CD57+	15.1 vs 13.2 %	p=.543	16.6 vs 10.3 %	p=.152	16.2 vs 16.2 %	p=.565	12.7 vs 16.2 %	p=.543	14.0 vs 17.7 %	p=.158	r=-.017	p=.887
	PD-1+ 2B4+	9.0 vs 10.0 %	p=.557	10.5 vs 6.4 %	p=.192	9.3 vs 13.8 %	p=.620	7.3 vs 10.7 %	p=.413	8.2 vs 11.5 %	p=.249	r=-.076	p=.527
	PD-1+ CD57+	12.1 vs 11.4 %	p=.983	13.5 vs 8.6 %	p=.167	12.7 vs 15.4 %	p=.843	10.9 vs 13.2 %	p=.629	**10.8 vs 14.7 %**	**p=.026**	r=.203	p=.089
	2B4+ CD57+	9.6 vs 9.3 %	p=.594	11.2 vs 5.6 %	p=.155	10.4 vs 13.1 %	p=.751	7.3 vs 11.0 %	p=.578	8.9 vs 11.1 %	p=.257	r=-.091	p=.453
	PD-1+ 2B4+ CD57+	6.3 vs 7.1 %	p=.706	7.4 vs 4.2 %	p=.260	6.6 vs 10.9 %	p=.634	5.0 vs 7.8 %	p=.303	5.8 vs 7.7 %	p=.268	r=-.051	p=.672
CD8+	PD-1+	52.6 vs 50.1 %	p=.554	54.1 vs 49.5 %	p=.179	52.0 vs 49.4 %	p=.661	50.7 vs 56.0 %	p=.421	**47.6 vs 59.1 %**	**p=.012**	**r=.233**	**p=.050**
	2B4+	75.8 vs 79.4 %	p=.546	77.7 vs 73.5 %	p=.339	77.3 vs 81.6 %	p=.500	74.7 vs 78.7 %	p=.407	74.7 vs 82.5 %	p=.176	r=.074	p=.539
	CD57+	52.9 vs 51.1 %	p=.718	54.2 vs 48.8 %	p=.207	54.4 vs 55.5 %	p=.872	50.7 vs 54.2 %	p=.639	51.5 vs 58.0 %	p=.229	r=.153	p=.203
	PD-1+ 2B4+	46.6 vs 45.0 %	p=.688	48.5 vs 42.8 %	p=.122	45.7 vs 45.2 %	p=.914	44.4 vs 50.4 %	p=.316	**41.9 vs 54.5 %**	**p=.003**	r=.227	p=.057
	PD-1+ CD57+	32.5 vs 29.5 %	p=.416	34.4 vs 27.9 %	p=.073	32.1 vs 30.7 %	p=.776	30.6 vs 34.6 %	p=.465	**28.3 vs 39.7 %**	**p=.003**	**r=.335**	**p=.004**
	2B4+ CD57+	53.3 vs 53.1 %	p=.973	55.3 vs 48.8 %	p=.153	54.5 vs 57.4 %	p=.674	51.2 vs 54.0 %	p=.713	51.6vs 59.5 %	p=.144	r=.137	p=.254
	PD-1+ 2B4+ CD57+	30.6 vs 27.6 %	p=.410	32.3 vs 26.3 %	p=.103	30.1 vs 28.8 %	p=.802	28.5 vs 32.6 %	p=.460	**26.8 vs 36.5 %**	**p=.011**	**r=.303**	**p=.010**