AMD3100 disrupts the cross-talk between chronic lymphocytic leukemia cells and a mesenchymal stromal or nurse-like cell-based microenvironment: pre-clinical evidence for its association with chronic lymphocytic leukemia treatments

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Online Supplementary Appendix

Reagent and antibodies

AMD3100 (A5602), fludarabine (F-Ara-A, F2773), cladribine (C4438), valproic acid (P4543) and favopiridol (F3055) were obtained from Sigma-Aldrich (Bornem, Belgium). Recombinant human SDF-1α was purchased from R&D Systems (Minneapolis, MN, USA). Methylprednisolone (Solu-medrol) was obtained from Pfeizer (Puurs, Belgium). Bortezomib was kindly provided by Janssen-Cilag, Belgium. Fludarabine, valproic acid, flavopiridol and bortezomib were dissolved in water. AMD3100 and SDF-1α were dissolved in phosphate-buffered saline (PBS). Cladribine was dissolved in PBS/ethanol (1:1). Stock solutions (1 mM) were stored at -20°C. Propidium iodide (PI), 3,3′-dihexyloxacarbocyanine iodide (DiOC6), annexin-V, and 7-aminoactinomycin D (7-AAD) were purchased from Invitrogen (Merelbeke, Belgium). The anti-CXCR4 (12G5, n. 551510) antibody was purchased from BD Biosciences Pharmigen (San Diego, CA, USA). All other cell surface monoclonal antibodies were purchased from Miltenyi Biotec (Bergish Gladbash, Germany). The Fix and Perm Permeabilization kit was obtained from Fisher Scientific (Erembodegem, Belgium). The vimentin (3B4 - M7020) antibody was purchased from Prosan (Merelbeke, Belgium), and the MCL-1 (22 – sc-12756) and FLIP (G11 – sc-5276) antibodies were purchased from Biosource (Nivelles, Belgium).

MNC from CLL patients culture condition

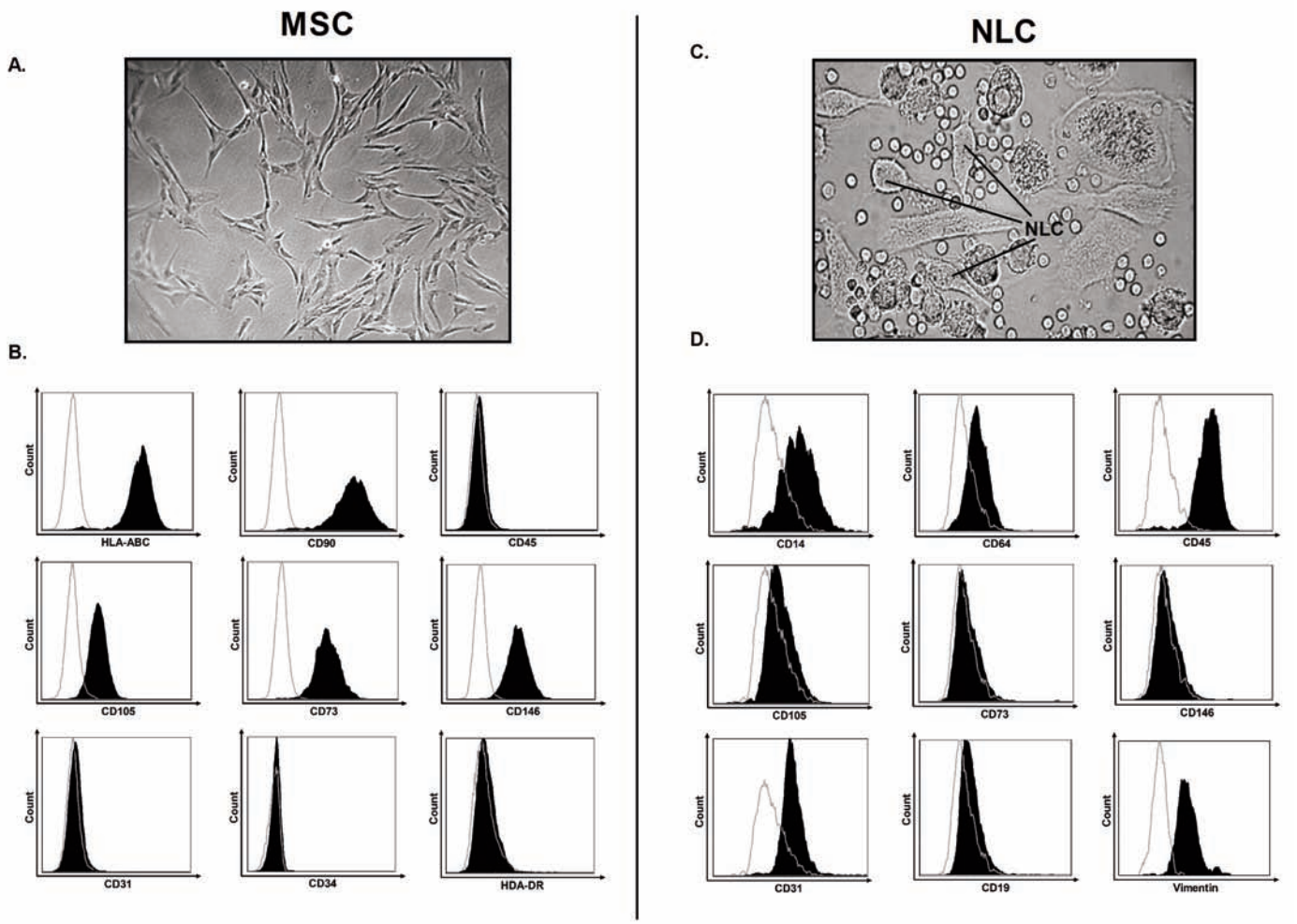
Mononuclear cells (MNC) of CLL patients were isolated from peripheral blood using density gradient centrifugation (Linfosep, Biomedics, Spain). The cells were immediately cultured (2×10^6 cells/mL) in RPMI 1640 (Lonza Europe, Verviers, Belgium) supplemented with streptomycin, penicillin, 2 mM L-glutamine and 10% fetal bovine serum (FBS) (Sigma-Aldrich) at 37°C in a humidified atmosphere containing 5% carbon dioxide.

Phenotypic analysis of MSC and NLC

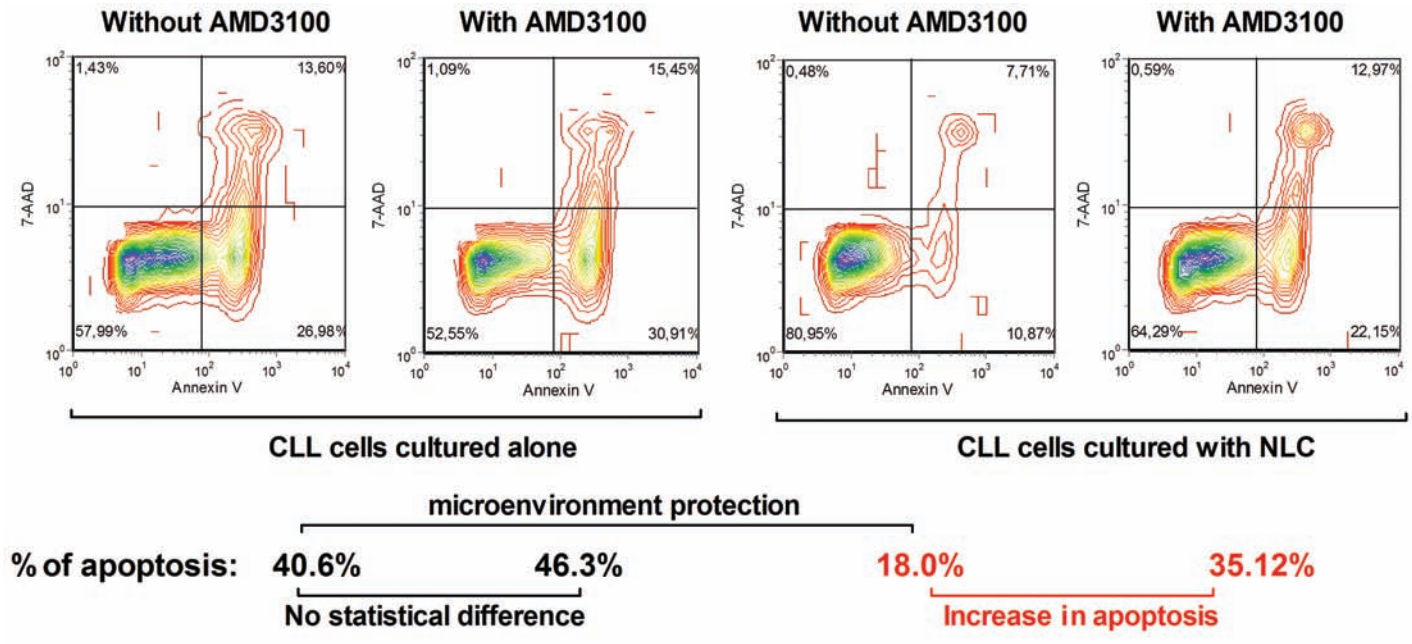
Phenotypic analysis was performed by flow cytometry using CD3, CD14, CD19, CD31, CD34, CD45, CD64, CD73, CD90, CD105, CD146, HL-DR, ABC, and vimentin monoclonal antibodies, as previously described.[1] Cytoplasmic vimentin level was determined in CD19-labeled cells using the Fix and Perm Permeabilization kit according to the manufacturer's recommendations. Results are shown in the *Online Supplementary Figure S1* and are in line with previous reports.[1,2]

References

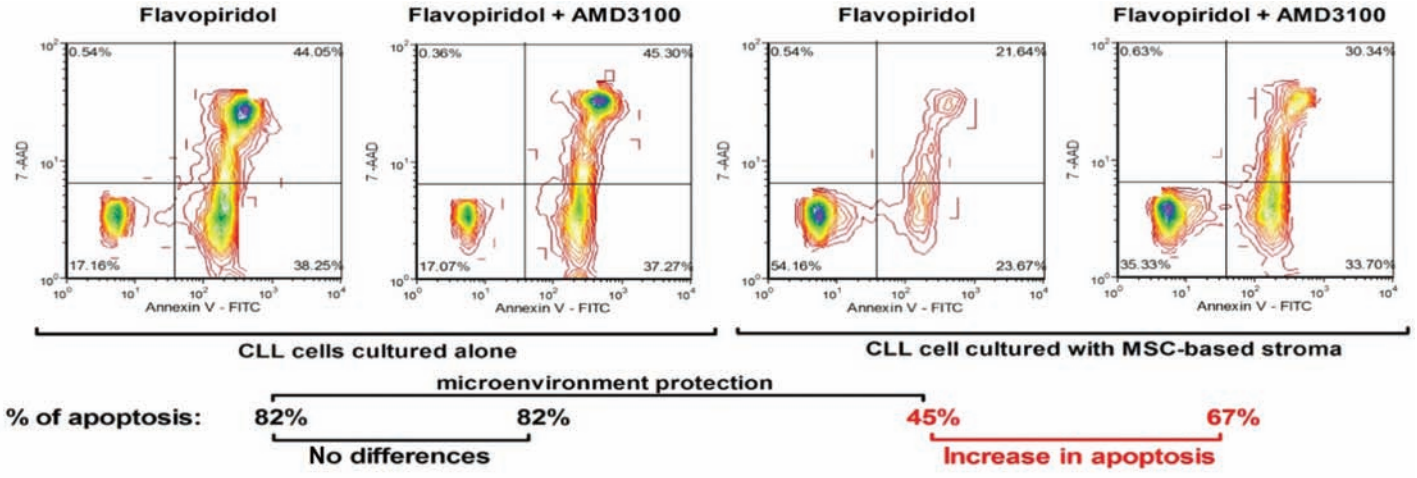
1. Najar M, Rouas R, Raicevic G, Boufker HI, Lewalle P, Meuleman N, et al. Mesenchymal stromal cells promote or suppress the proliferation of T lymphocytes from cord blood and peripheral blood: the importance of low cell ratio and role of interleukin-6. Cytotherapy. 2009;11(5):570-83.
2. Burger JA, Tsukada N, Burger M, Zvaifler NJ, Dell'Aquila M, Kipps TJ. Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1. Blood. 2000;96 (8):2655-63.



Online Supplementary Figure S1. Morphological and phenotypical characterizations of MSC and NLC. Photography of MSC (A) and NLC (C). Phenotypic characterization of MSC (C) revealed that MSC are negative for hematopoietic markers (CD34, CD45), for endothelial marker (CD31) and positive for putative mesenchymal markers (CD105, CD73, CD146, CD90). MSC are negative for HLA-DR but positive for HLA-ABC. Phenotypic characterization of NLC (D) revealed that NLC are positive for hematopoietic markers (CD45), for monocyte/macrophage marker (CD64, CD14), for endothelial marker (CD31) and negative for B-cell marker (CD19) and putative mesenchymal markers (CD105, CD73, CD146). NLC are also positive for vimentin.



Online Supplementary Figure S2. AMD3100 restores apoptosis in presence of an MSC-based or NLC-based microenvironment. Representative annexin V/7-AAD staining on CD19$^+$ cells with/without AMD3100 and with/without NLC. In absence of NLC, AMD3100 did not have a statistical effect on apoptosis. In presence of NLC, spontaneous apoptosis of MNC from CLL patients was reduced but AMD3100 partially restores this apoptosis.



Online Supplementary Figure S3. AMD3100 sensitizes MNC from CLL patients to drug-induced apoptosis in presence of an MSC-based microenvironment. Representative annexin V/7-AAD staining on CD19$^+$ cells with/without AMD3100, with/without flavopiridol and with/without MSC. In absence of MSC, AMD3100 did not have a statistical effect on drug-induced apoptosis. In presence of MSC, drug-induced apoptosis of MNC from CLL patients was reduced but AMD3100 partially restores this apoptosis.

Online Supplementary Table S1. Clinical characteristics of chronic lymphocytic leukemia patients. UM: unmutated; M, mutated; ND: not done. IGHV mutational status and ZAP70 are based, respectively, on 98% and 20% cut-off values (commonly accepted in the literature). Optimal cut off for CD38, determined by ROC curve analysis, was 7%. Cytogenetic abnormalities were investigated by conventional karyotype analysis and a CLL FISH panel detecting del(17p), del(11q), del(6q), del(13q) and trisomy 12.

	Age (years)	Sex	Binet stage	White blood cell count ($x10^9$/L)	CD5/CD19 (%)	IGHV mutational status	ZAP70	CD38	Cytogenetic abnormalities
1	55	M	A	68	90	UM	+	+	del(13q-) del (11q)
2	51	F	B	138	95	UM	+	+	del(11q)
3	45	F	A	193	99	UM	+	-	del(13q)
4	75	M	A	36.2	75	M	-	+	trisomy 12
5	77	M	A	34	75	M	-	+	del(13q)
6	69	M	A	25.3	80	M	-	-	del(13q)
7	62	M	A	145.8	94	M	+	+	del(13q)
8	76	M	A	108.7	76	UM	+	+	ND
9	59	M	A	32.9	90	M	-	-	ND
10	53	F	A	115	96	M	-	+	ND
11	73	M	A	20.7	72	M	-	+	normal
12	72	F	A	71.2	56	M	-	-	normal
13	46	F	B	78	94	UM	+	-	del(13q)
14	65	M	C	60.7	91	M	-	+	del(6q)
15	78	M	B	35.8	79	UM	+	+	trisomy 12
16	62	M	A	22.1	89	M	-	-	del(16q)
17	68	M	A	74.4	73	UM	+	+	del(11q)
18	72	F	A	25.4	75	M	-	-	del(17q)
19	52	F	A	14.8	76	M	-	-	normal
20	48	F	A	25.7	90	M	-	-	del(13q)
Summary	64.3	12 M 8 F	17 A 3 B 1 C	71.4	83.3	7 UM 13 M	8 + 12 -	11 + 9 -	7 unfavorable 9 favorable 3 ND