SUPPLEMENTAL MATERIAL

Expanded and activated allogeneic NK cells are cytotoxic against B-chronic lymphocytic leukemia (B-CLL) cells with sporadic cases of resistance.

by

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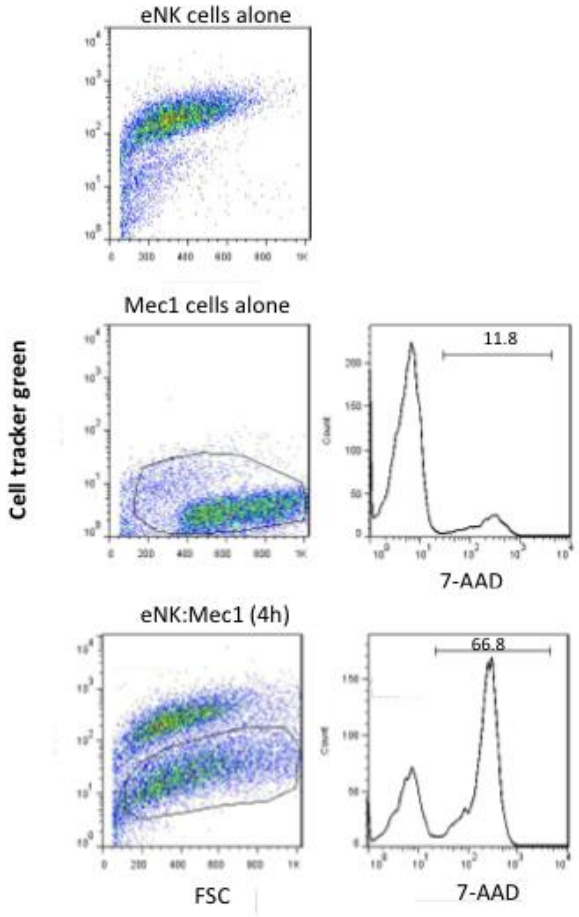
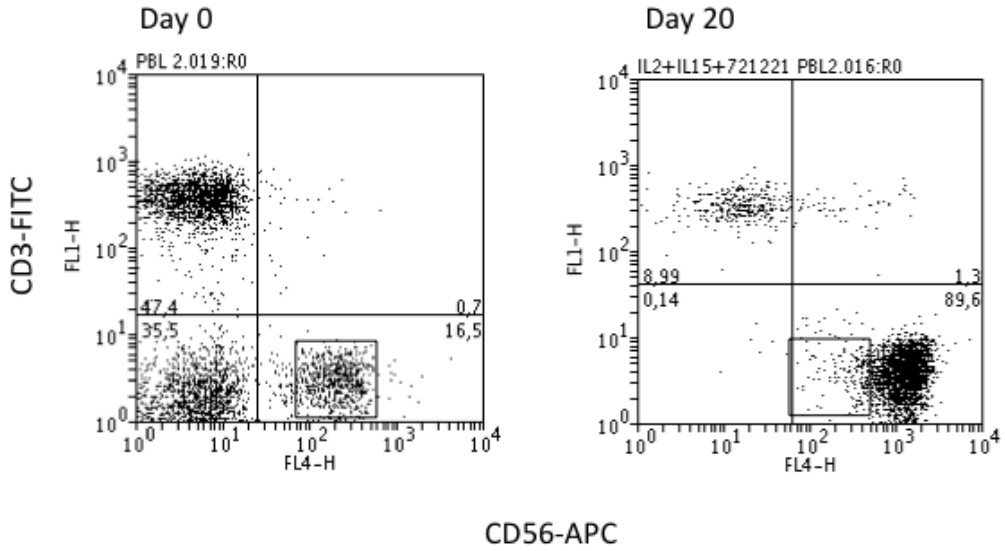
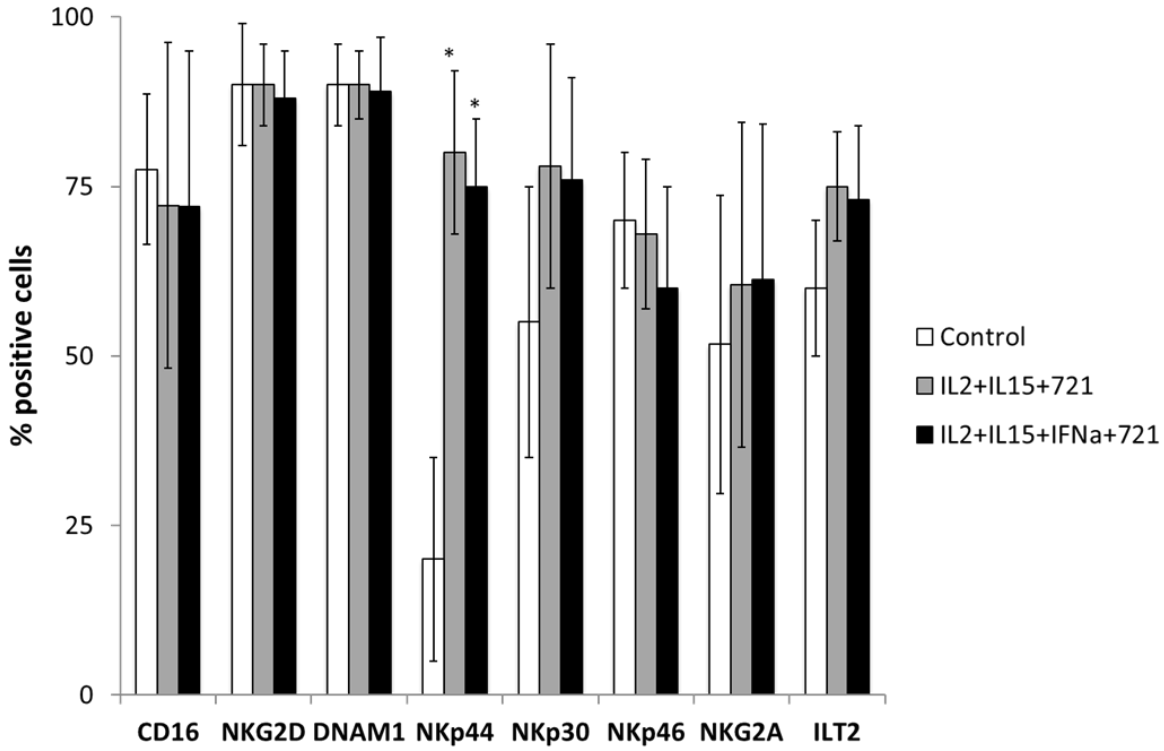


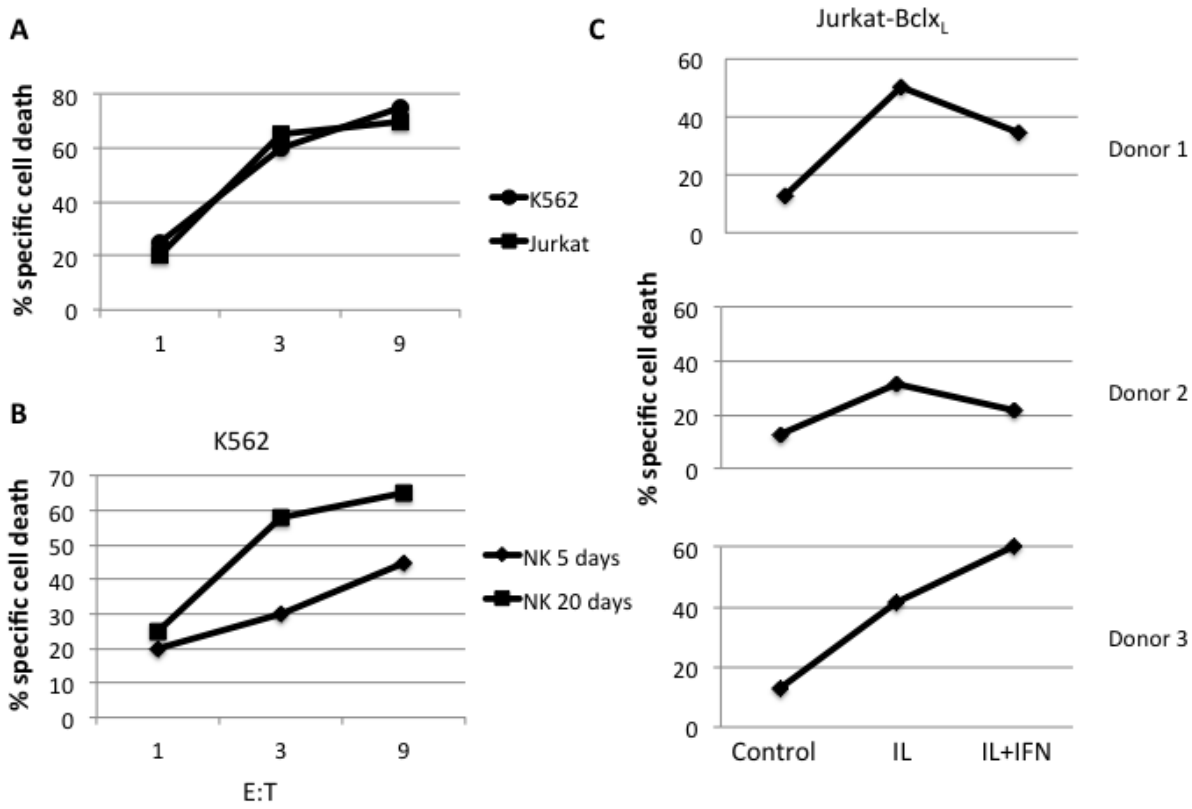
Supplemental Figure 1. Example of a representative cytotoxicity assay using eNK cells against leukemic Mec1 cells. eNK cells were labeled with Cell Tracker Green (CTG), and this labeling alone is shown in the upper dot plot vs. FSC. Mec1 cells were not labeled with CTG and analyzed at time 0 vs. FSC (middle dot plot). This was used for gating cells and to analyze basal Mec1 cell death on the gated population by 7-AAD staining (middle histogram). eNK cells were mixed at a 1:1 E:T ratio and the cytotoxicity assay developed during 4h. After this time, CTG labeling was analyzed on the mixed population vs. FSC, allowing gating of the Mec1 population (lower left dot plot). This gating was used to analyze cell death induced by eNK cells on Mec1 cells by 7-ADD staining (lower histogram).



Supplemental Figure 2. Example of the variation in the CD56 phenotype in eNK cells (NK3; day 20) with respect to NK cells at day 0; at day 0, most NK cells are $CD56^{dim}$ (squared population), while at day 20, most NK cells are $CD56^{bright}$ and are outside the square.



Suppl. Figure 3. Phenotype of expanded NK (eNK) cells. Percentage of NK cells positive for the expression of the indicated surface receptors at day 0 (white bars), and after 20-day expansion following the protocol indicated in Material and Methods in the presence (IL2+IL15+IFN□+721, black bars) or in the absence of IFN□α□(IL2+IL15+721, grey bars). Data are the mean ± SD of data obtained in cells from the same 10 donors used in the expansion experiments (Supplemental Table I) and in the cytotoxicity assays shown in Fig 2A, except NK5 and NK6. Significance was determined by Student's t-test; *, *P*<0.05.



Supplemental Figure 4. eNK cells were labeled with cell tracker green (CTG) and tested against different leukemic target cells for 4h at the E:T ratios indicated. Then, target cells were gated as shown in the previous Figure, and cell death was tested by nuclear 7-AAD incorporation. Results are shown as percentage of specific cell death induction, subtracting basal cell death, which was never higher than 15%. A, expanded NK cells were tested at the indicated E:T ratios against HLA-I negative K562 or against HLA-I positive Jurkat cells; B, NK cells activated for 5 or for 20 days, as indicated, with IL-2+IL-15+721.221 feeder cells, were tested on K562 target cells at the indicated E:T ratios; C, non-activated NK cells (control), or eNK cells generated in the presence of IL-2 plus IL-15 (IL) or in the presence of IL-2, IL-15 and IFN-α (IL+IFN) from three different donors were tested at a 5:1 E:T ratio against Jurkat cells over-expressing the anti-apoptotic molecule Bcl-x_L (Jurkat-Bcl-x_L).

CODE	AGE	HEMOGRAME (HGB,LK(Ly),PL)	STAGE AT DIAG.	TREATMENT (TT)	TIME UNTIL TT	RESPONSE	DURATION OF RESPONSE	GENETICS	ZAP70	CD38	STAGE AT 21/02/2017
CLL 001	52	15,9/10,100(6,400)/263,000	IB	/	/	/	/	DELETION 11q22-q23 (ATM gene)	/	54	B. PROGRESSION
CLL 01	72	13,1/23,49(18)/178	IIA	Fluda, Chlor, R-Chlor	4y	Complete	5y	N.D.	26	/	EXITUS
CLL 1	77	11,2/232.000/112	0	2012 rituximab + chlorambucil.	10y	Partial	18m	N.D.	/	5	IVC.PROGRESSION.
CLL 2	89	5/140.000(133.000)/134	IIIC	Chlorambucil + Prednisone	5m	Partial	In treatment	48XX +12 +21	/	/	EXITUS
CLL 3	66	11,1/1,9(1)/100	IIIC	Prednisone, R-Benda x1, COP x2	6y	No	In treatment	46xy T(2;14)	32	7	IIIC. PROGRESSION
CLL 4	74	12,4/40,8(36)/109	0	/	/	/	/	N.D.	/	20	0. W/O TT.
CLL 5	75	12,7/114/122	0	R-COP x6, R-Benda x1	3y	Partial	3y	FISH: del(13q) heterozygosis(84%)	10	70	IVC.PROGRESSION.IN TT.
CLL 06	58	12,2/155,000/65	IA	FC x4, R-FC x6, R-Benda x6	6m	Complete	3y	del 11(q22.3)	67	73	IA. 13,9/127(121,7]/125
CLL 6	74	15,2/49,7(42,3)/179	0	/	/	/	/	IgVH Mut	2	neg	IA.14,9/52,2(43,6]/224
CLL 7	75	8,7/279(250)/113	IA	R-FC x6, R-Benda x1	13	Complete	3y	N.D.	6	18	IVC.PROGRESSION.IN TT.
CLL 8	89	11,2/40,2(35,3)/174	0	/	/	/	/	N.D.	/	/	0. W/O TT.11,2/40,2(35,5]/174
CLL 9	81	12,6/4(2,3)/76	IA	Chlorambucil, Ritu-Prednisone	8y	Partial	In treatment	N.D.	/	neg	IIA. PROGRESSION
CLL 10	85	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.D.	/	/	IA. 12,6/126,8[119,1]/113
CLL 11	80	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.D.	/	/	0. W/O TT. 12,5/30,6(25,5]/171
CLL 012	63	12,1/2,2(0,1)/165	0	Ig-Prednisone RFC	7y	Complete	In treatment	del 13q (33%) ATM + (21%)	4	/	IV. PROGRESSION.
CLL 12	83	9,3/19,7(18,6)/27	IIIA	Chlorambucil, R-chlorambucil	1y	Partial	2y.In TT	N.D.	/	/	IV.PROGRESION.9,3/19,7(18,6]/27
CLL 13	44	15,6/125,1[118,5]/154	0	/	/	/	/	del 13q	/	neg	0. W/O TT. 15,5/125,1[118,5]/154
CLL 14	77	11,3/6,3(3,8)/43	0	R-chlorambucil	14y	/	In treatment	del 13q	/	neg	IVC. IN TT. 11,3/6,3(3,8]/43
CLL 15	66	11,6/17(12,6)/36	0	Rituximab, RFC	1y	Partial	In treatment	del (13q14.3)	/	49	IN TREATMENT
CLL 16	77	15/20,4(16,3)/109	0	/	/	/	/	N.D.	/	neg	0.W/O TT.15/20,4(16,3]/109
CLL 17	71	14,8/13,9(10,4)/168	0	/	/	/	/	N.D.	/	/	0.W/O TT.14,8/13,9(10,4]/168
CLL 0000018	71	N.A.	0	/	/	/	/	/	/	neg	0. W/O TT. 15.1/17(13,6]/184
CLL 000018	61	14/9,8(4,9)/224	0	/	/	/	/	/	/	neg	0. W/O TT. 14/9,8(4,9]/224
CLL 00018	82	12,4/49,5(45)/142	0	/	/	/	/	N.D.	/	/	0. W/O TT. 12,4/49,5(45]/142
CLL 0018	79	13,4/23,8(20,4)/123	0	/	/	/	/	46XX.Del 13(q)	1,6	neg	0.W/O TT.13,4/23,8(20,4]/123
CLL 6 (2016)	BIS	BIS	BIS	R-Bendamustine	BIS	BIS	BIS	BIS	BIS	BIS	C. PROGRESSION.
CLL 5 (2016)	BIS	BIS	BIS	BIS	BIS	BIS	BIS	BIS	BIS	BIS	IN TT.12,2/219,2(205]/121
CLL 18	76	9,2/310(292)/59	0	R-Benda	11y	Complete	2y	46 XY. Del (13q) heterozygosis	0,05	1,5	IVC. PROGRESSION
CLL 19	89	13,4/32(13,6)/190	0	/	/	/	/	N.D.	/	/	0. W/O TT. 13,4/32(13,6]/190
CLL 20	87	12,2/6,9(1,4)/22	0	Leukeran	3y	In TT	/	ATM mut. IgVH mut.	/	/	IIB W/O TT. 14,6/143(130]/133
CLL 21	76	15,5/14,9(8,8)/166	0	/	/	/	/	N.D.	/	/	0.W/O TT. 14,1/21,3(15,8]/194
CLL 8 (2016)	BIS	BIS	BIS	BIS	BIS	BIS	BIS	BIS	BIS	BIS	0.W/O TT.11,5/18,2(14,5]/166
CLL 22	74	10/39,2(34,3)/198	0	/	/	/	/	N.D.	/	/	0.W/O TT.10/39,2(34,3]/198

Supplemental Table I (previous page). Clinical data of the 30 patients enrolled in the study. Hemograme expressed as hemoglobin value in g/dL/number of leukocytes x 10^3/µL (of which number of lymphocytes x 10^3/µL)/number of platelets x 10^3/µL; Fluda,fludarabine; Chlor, chlorambucil; R-Chlor, rituximab plus chlorambucil; R-COP, rituximab plus cyclophosphamide, vincristine and prednisone; R-Benda; rituximab plus bendamustine; FC, fludarabine plus cyclophosphamide; R-FC, rituximab plus fludarabine and cyclophosphamide. Genetics performed: karyotype, deletion of chromosome 13, deletion of chromosome 11q (ATM gene), trisomy of chromosome 12, deletion of chromosome 17 (17p) and IgVH mutation analysis.

			mean
IL2+IL15+721.221	Donor 1 (NK1) –	34	58
	Donor 2 (NK2) –	32	
	Donor 3 (NK3) –	33	
	Donor 4 (NK4) –	42	
	Donor 5 (NK5) –	30	
	Donor 6 (NK6) –	-	
	Donor 7 (NK7) –	195	
	Donor 8 (NK8) -	32	
	Donor 9 (NK9) -	30	
	Donor 10 (NK10) -	91	
IL2+IL15+IFNα+721.221	Donor 1 (NK1) –	21	61
	Donor 2 (NK2) –	16	
	Donor 3 (NK3) –	61	
	Donor 4 (NK4) –	67	
	Donor 5 (NK5) –	55	
	Donor 6 (NK6) –	50	
	Donor 7 (NK7) –	121	
	Donor 8 (NK8)-	55	
	Donor 9 (NK9) -	16	
	Donor 10 (NK10) -	144	

Supplemental Table II. Expansion of NK cells following the two protocols tested, expressed as fold expansion with respect to time 0.

	Age	Stage	Treatment	Response	%CD38	%ZAP70
CLL 5	75	IA (2015)	R-COP x6 R-Benda x1	Partial	70	10
		IV-C (2016)	R-Benda	In treatment	Bis	Bis
CLL 8	**89**	0 (2015)	No	·	0	·
		0 (2016)	No	·	Bis	Bis

Supplemental Table III. Clinical data of patients 5 and 8 at the time of the first test (2015) and at the time of the second test (2016) with eNK cells