HLA-G is a component of the chronic lymphocytic leukemia escape repertoire to generate immune suppression: impact of the *HLA-G* 14 base pair (rs66554220) polymorphism

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Supplementary Table 1. Demographic, clinical, laboratory and molecular variables of the CLL population subdivided according to rs 66554220 polymorphism.

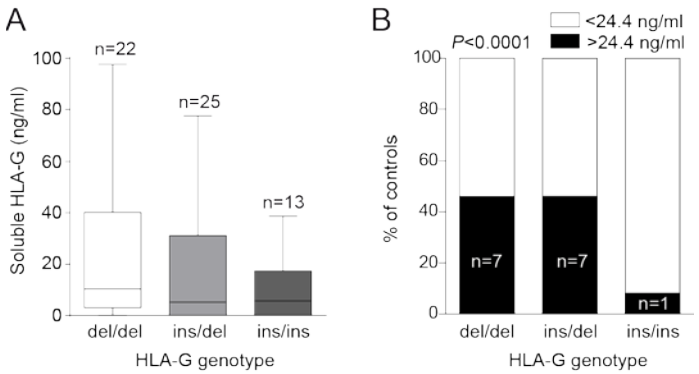
		HLA-G ins/ins n(%)	HLA-G ins/del n(%)	HLA-G del/del n(%)	Total	p value
CD38	≤30%	40 (49)	158 (63)	108 (61)	306	
	>30%	41 (51)	91 (37)	68 (39)	200	0.076
	Total	81	249	176	506	
ZAP70	≤20%	42(52)	132 (53)	96 (55)	270	
	>20%	39 (48)	117 (47)	80 (45)	236	0.19
	Total	81	249	176	506	
FISH	13q-/normal/+12	54 (67)	157 (63)	118 (67)	368	
	17p-/11q-	27 (33)	92 (37)	58 (33)	138	0.66
	Total	81	249	176	506	
***IGHV* homology**	≤98%	40 (49)	157 (63)	104 (59)	301	
	>98%	41 (51)	92 (37)	72 (41)	205	0.09
	Total	81	249	176	506	
Age (years)	<65	31 (38)	100 (40)	64 (36)	195	
	≥65	50 (62)	149 (60)	112 (64)	311	0.73
	Total	81	249	176	506	
Binet Stage	A	51 (63)	190 (76)	125 (71)	366	
	B - C	30 (37)	59 (24)	51 (29)	140	0.06
	Total	81	249	176	506	
Sex	F	41 (51)	100 (40)	73 (41)	214	
	M	40 (49)	149 (60)	103 (59)	292	0.25
	Total	81	249	176	506	
Lymphocytes	$<15x10^9/l$	50 (62)	132 (53)	97 (55)	279	
	$>15\ x10^9/l$	31 (38)	117 (47)	79 (45)	192	0.39
	Total	81	249	176	506	
Lynph Node Size	≤3cm	71 (88)	198 (79)	143 (81)	412	
	>3cm	10 (12)	51 (21)	33 (19)	94	0.26
	Total	81	249	176	506	
Splenomegaly	No	52 (64)	190 (76)	134 (76)	386	
	Yes	29 (36)	59 (24)	42 (24)	120	0.075
	Total	81	249	176	506	
B2M	<2.5 mg/l	44 (54)	100 (40)	82 (47)	226	
	>2.5 mg/l	37 (46)	149 (60)	94 (53)	280	0.07
	Total	81	249	176	506	
LDH	<500 U/l	50 (62)	210 (84)	145 (82)	405	
	>500 U/l	31 (38)	39 (16)	31 (18)	101	0.11
	Total	81	249	176	506	

The genotypes ins/ins and ins/del were considered together as low and medium HLA-G producers and compared with the del/del high HLA-G producer genotype.

FISH, fluorescence in situ hybridization; *IGHV*, immunoglobulin heavy variable gene; B2M, beta-2-microglobulin.

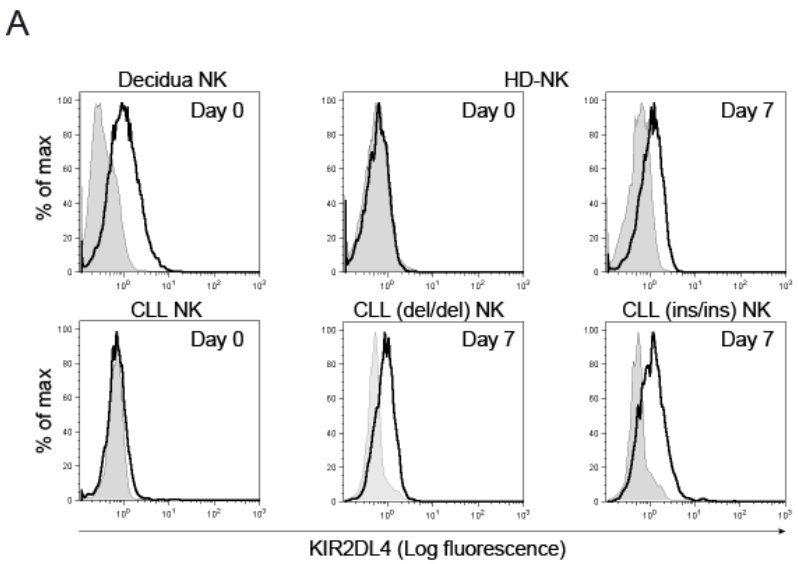
p values were obtained by chi square test or Fisher's exact test, when appropriate.

Supplementary Figure 1. **Quantification of soluble HLA-G levels in a cohort of 60 controls typed for the 14 bp polymorphism. (A)** Graph showing soluble HLA-G levels in control subjects divided according to the 14 bp polymorphism into del/del, ins/del and ins/ins categories. (**B**) Graph representing the percentage of control subjects expressing soluble HLA-G above (black bars) or below (open bars) the third quartile (24.4 ng/ml) in the three genotypes.



SUPPLEMENTARY FIGURE 1

Supplementary Figure 2. Phenotypic analysis of KIR2DL4 on NK cells. (**A**) NK cells from decidual tissue (dNK), healthy donor (HD-NK) and 2 representative CLL patients were analyzed for surface expression of KIR2DL4 at day 0 and after 7 days of culture in IL-2. Staining with secondary antibody alone is shown (gray profile). One representative experiment out of 10 performed.



SUPPLEMENTARY FIGURE 2

Supplementary Materials and Methods

Antibodies for flow cytometric analyses

Antibodies used were anti-HLA-G-PE (clone 87G), -CD19-FITC, -CD5-FITC, -CD4-PE-Cy5 (all from eBioscience, Milan, Italy), -CD38-PE (EXBIO Praha, Vestec, Czech Republic), -ZAP-70-AlexaFluor488 (Life Technologies, Invitrogen, Monza, Italy), -CD8-FITC (Biolegend, Milan, Italy), -CD3-PerCP, -CD56-APC, -CD19-APC (Miltenyi Biotech, Calderara di Reno, Italy). KIR2DL4 (R&D Systems, Abingdon, UK) was highlighted using a PE-conjugated rabbit anti-mouse secondary antibody (Southern Biotechnologies, Birmingham, AL). Tregs were detected using anti-CD4-PerCP/-CD25-PE/-CD127-AlexaFluor647 mix (Biolegend).