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Abstract: The human leukocyte antigen-G (HLA-G) gene encodes a tolerogenic protein known to promote tumor immune-escape. We investigated HLA-G polymorphisms and soluble molecules (sHLA-G) in 68 chronic myeloid leukemia (CML) patients. Patients with G*01:01:01 or G*01:01:02 allele had higher value of sHLA-G compared to G*01:01:03 (109.2±39.5 vs 39.9±8.8 units/ml; p=0.03), and showed lower event free survival (EFS) (62.3% vs 90.0%; p=0.02). The G*01:01:03 allele was associated with higher rates and earlier achievement of deep molecular response (MR)4.5 (100% vs 65%, median of 8 vs 58 months, p=0.001). HLA-G alleles with higher secretion of sHLA-G seem associated with lower EFS, possibly because of a inhibitory effect on the immune system. Conversely, lower levels of sHLA-G promoted achievement of MR4.5, suggesting increased cooperation with immune system.

Revision note

Dear Editor, we have carefully evaluated the revisions requested by reviewers and provided a point-by-point answer. Changes in main text are in red color.

Reviewer #1:

1.*The Introduction provide a rationale for the study, starting with the role of HLA-G expression in onset and progression of cancer (para 2); interference in tumor immune-editing (para 3); and prior investigations in other hematological malignancies and HSCT (para 4); leading to the rationale for their study (para 4). It would be helpful, however, to elaborate more on the hypothesis driving the study - as further rationale for the study but also to resume on in the Discussion section.*

Answer. We added the following statement in the paragraph 4: "In particular, in patients presenting HLA-G polymorphism associated with lower levels of sHLA-G, NK cells could cooperate more actively in the achievement of biological recovery through a better control on cancer cells; conversely, patients with high secretor genetic profile and likely with NK inhibition, would be disadvantaged. On these bases, HLA-G could represent a immune biomarker predictive of deeper response to TKI therapy and could help in the identification of CML patients who are likely to benefit from a program of TKI discontinuation. Additionally, it may represent an innovative target for immune-mediated treatment approaches to CML. To investigate this hypothesis…"
We also resumed it in the discussion section.

2.*The statistical analyses are, essentially, uncontrolled for potential covariates or confounders. Such information may not have been collected, and we cannot expect the authors to correct this retroactively given the preliminary, exploratory nature of their study. However, it would be helpful to speculate about this in the Discussion to guide future research along this line and, eventually, potential translation to the clinic.*

Answer.
We added the following statement at the end of discussion: "Given the retroactive preliminary and exploratory nature of our study, statistical analysis was not adjusted for potential covariates or confounders. Further studies on wider cohort of patients and adjusted for confounding effects, will be required to confirm our findings".

3.*Where and how do the authors see their work evolving? What are the next (specific) studies that they plan?*

Answer. We have preliminary results on a small cohort of 24 CML patients that discontinued TKI. HLA-G profiles seem to confirm our preliminary hypothesis even in this setting of patients. Indeed, treatment free remission rate seems to be associated with lower expression of HLA-G. Next step will be to confirm these data in a wider cohort of patients discontinued from TKI. We have addressed the issue about a possible correlation between HLA-G and TKI discontinuation both in introduction and discussion section.

4.*In the Discussion section, some further discussion of the underlying hypothesis, mechanisms, etc. would be helpful.*

Answer. We added 2 statements in the discussion section: "Different to what has been observed in solid tumors, B and T malignant cells express receptors recognized by HLA-G molecules. Hence, the role played by HLA-G in oncohematologic diseases is probably more complex" and "Experience in hematopoietic stem cell transplantation (HSCT) and treatment of CML recurrence after HSCT by donor lymphocyte infusion (DLI) has considerably contributed to our understanding of the role of cytotoxic lymphocytes and NK cells in the control of leukemia stem cells and achievement of biological recovery"

5.*It seems essential to state the preliminary and exploratory nature of the work reported here*.

Answer. See answer to point 2

Reviewer #2:

1. *Concerning reports of prognostic markers in CML, it is customary to include an attempt at multivariate analysis to allow the reader to assess the importance and utility of the novel biomarker in relation to those already known, such as Sokal, treatment assigned, Early Molecular Response etc (unless a biological mechanism for the observed effect has been demonstrated in the same manuscript, which is not the case here).*
2. *It is also customary to perform cox proportional hazard models at a univariate level*.

Answer. We performed a binary logistic regression analysis.
In the statistical analysis section we added: "All independent variables studied (Sokal risk, age >45 years, frontline 1st generation TKI vs 2nd generation, numbers of TKI lines, $MR^{4.5}$ achievement by 18 months, HLA-G polymorphism in/in or in/del or del/del, number of HLA-G 01:01:01 or 01:01:02 or 01:01:01 alleles, sHLA-G low, intermediate or high secretion) were included in a binary logistic regression analysis, where ESF status and $MR^{4.5}$ achievement were considered as dependent variables. Variables with a p-value lower than 0.2 in univariate analysis were analysed in a multivariate level with a model=enter procedure, employed to find the best equation. Only p-values ≤ 0.05 were considered to be statistically significant".
In the results section we added: "The multivariate analysis showed that $MR^{4.5}$ achievement was negatively correlated with frontline 1st generation TKI (p=0.018; OR=0.024, 95% C.I.= 0.01-0.53) and HLA-G 01:01:02 allele (p=0.047; OR=0.11, 95% C.I.= 0.11-0.97). EFS was significantly associated with higher sHLA-G in univariate (p=0.05) but not multivariate analysis".

3. *The rates of molecular response should be presented using time dependent analyses, such as the cumulative incidence function. The text indicates (on page 8) that this has been included as figure 1, though figure 1 is a K-M curve for EFS*

Answer. It was merely a mistake and reference to Figure 1 on page 8 was shifted in the right position (after EFS statement).

4. *The authors suggested that the "G*01:03 allele was significantly associated to CML onset", although I suspect that they merely mean that their sample has an over-representation of G*01:03 allele compared to the general population as represented in the 1000Genome project? Does this have the potential to affect outcomes of the study?*

Answer. We changed the sentence "Only G*01:03 allele was significantly associated to CML onset" according to your suggestion: "Only the G*01:03 allele was over-represented in comparison with the referring population".

5.*EFS is a composite end point - the number of events in each category should be supplied*

Answer. The number of events in each category was added in the result section

6. *Rates of major molecular response should be supplied*.

Answer. We added in the results section: "The 10-year cumulative incidence of MR^{3} and $MR^{4.5}$ was 83.6% and 72.5%, respectively".

Highlights

- HLA-G molecules play a role in cancer immune-editing and NK cell inhibition
- Lower event free survival in CML is associated to higher level of HLA-G
- Deeper molecular response in CML is associated to lower level of HLA-G
- HLA-G could represent a new useful predictive immune biomarker in CML
- HLA-G is a innovative target for immune-mediated treatment approaches

HLA-G molecules and clinical outcome in Chronic Myeloid Leukemia

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Abstract

The human leukocyte antigen-G (HLA-G) gene encodes a tolerogenic protein known to promote tumor immune-escape. We investigated HLA-G polymorphisms and soluble molecules (sHLA-G) in 68 chronic myeloid leukemia (CML) patients. Patients with G*01:01:01 or G*01:01:02 allele had higher value of sHLA-G compared to G*01:01:03 (109.2±39.5 vs 39.9±8.8 units/ml; p=0.03), and showed lower event free survival (EFS) (62.3% vs 90.0%; p=0.02). The G*01:01:03 allele was associated with higher rates and earlier achievement of deep molecular response ($MR^{4.5}$) (100% vs 65%, median of 8 vs 58 months, p=0.001). HLA-G alleles with higher secretion of sHLA-G seem associated with lower EFS, possibly because of a inhibitory effect on the immune system. Conversely, lower levels of sHLA-G promoted achievement of $MR^{4.5}$, suggesting increased cooperation with immune system.

Introduction

The human leukocyte antigen-G (HLA-G) is a non-classical HLA class I gene on chromosome 6p21.3 of the major histocompatibility complex (MHC) that encodes tolerogenic proteins with a crucial role in fetal-maternal tolerance. HLA-G molecules are primarily expressed at the fetal-maternal interface, but are also present in other tissues and cells such as adult thymus, pancreatic islets, cornea, mesenchymal cells, monocytes and precursors of endothelial and erythroid cells.

HLA-G expression has been suggested to have an important role in infections and the onset and progression of cancer [1]. Unlike the highly polymorphic classical HLA-class I genes, HLA-G exhibits low levels of polymorphism. So far, 53 alleles, 18 full-length proteins, and 2 null alleles have been identified. Alternative splicing can generate 7 different HLA-G isoforms: 4 membrane isoforms (G1-G4) and 3 soluble (s) isoforms (G5-G7) [2]. Higher or lower secretion of sHLA-G would seem to be associated to different HLA-G coding haplotypes that have recently been described within a worldwide genome analysis project [3]. Also allelic variants characterized by a 14-basepair (14-bp) deletion-insertion polymorphism located in exon 8 of the 3'-untranslated region (3'UTR) of *HLA-G* have been associated to different mRNA stability and sHLA-G levels, both in normal and pathological conditions [4]. HLA-G molecules exert their tolerogenic activity through interaction with the immunoglobulin (Ig)-like transcript 2 (ILT2) and ILT4 inhibitory receptors expressed on natural killer (NK) cells, T and B lymphocytes, dendritic cells and neutrophils. For this reason, HLA-G/ILT interaction is considered an emerging immune checkpoint [1].

HLA-G can interfere in all phases of tumor immune-editing (elimination, equilibrium and escape) and has been associated to tumor progression and unfavorable outcome or prognosis [5,6,7,8]. A correlation between HLA-G expression, tumor onset and clinical outcome has been investigated in multiple myeloma (MM), non-Hodgkin B-

lymphoma (NHL-B), Hodgkin lymphoma (HL), B-cell chronic lymphocytic leukemia (B-CLL) and acute leukemia [9-14]. Increased sHLA-G plasma levels have been found in MM, NHL-B and B-acute or chronic leukemia [9-11]. The impact of HLA-G expression has also been evaluated in allogeneic hematopoietic stem cell transplantation (HSCT) [15-17].

To the best of our knowledge, there are no studies in the literature on association of HLA-G expression and the clinical outcome of chronic myeloid leukemia (CML). The innate immune system is likely to have an important role in the achievement of therapeutic response and recovery through control or eradication of quiescent CML stem cells during Tyrosine Kinase Inhibitory (TKI) treatment [18,19]. We identified a potential role for Natural Killer (NK) cells in the achievement of deeper molecular remission (MR) during TKI treatment [20]. Given that HLA-G has been reported to inhibit NK function and interferon-γ secretion through ILT2 receptors expressed on the NK cell membrane [1], we hypothesized a possible implication of these molecules in the modulation of therapeutic response to TKIs. In particular, in patients presenting HLA-G polymorphism associated with lower levels of sHLA-G, NK cells could cooperate more actively in the achievement of biological recovery through a better control on cancer cells; conversely, patients with high secretor genetic profile and likely with NK inhibition, would be disadvantaged. On these bases, HLA-G could represent a immune biomarker predictive of deeper response to TKI therapy and could help in the identification of CML patients who are likely to benefit from a program of TKI discontinuation. Additionally, it may represent an innovative target for immune-mediated treatment approaches to CML.

To investigate this hypothesis, we analyzed allelic polymorphisms in the coding region and the 3'UTR of the HLA-G gene and assessed sHLA-G serum levels in a cohort of 68 CML patients. We then investigated the possible association of HLA-G haplotypes and sHLA-G expression levels with achievement of deep MR and event free survival (EFS).

Patients and methods

Sixty-eight chronic-phase CML patients attending 4 Italian institutions, were enrolled in the study. Written informed consent was provided by the patients according to the declaration of Helsinki. The study was approved by the local ethics committee of the participating institutions. Cytogenetic testing was performed at diagnosis, 6 and 12 months after starting treatment and subsequently, once a year. Real-time (RT) PCR for BCR-ABL transcripts was performed every 3 months for the first 2 years, and every 6 months thereafter. Deeper MR was defined by ≥4-log reductions in BCR-ABL transcript level. Complete molecular remission (CMR) was defined by the absence of detectable BCR-ABL transcripts using qRT-PCR with a sensitivity of 4.5 logs ($MR^{4.5}$). EFS was defined considering as event the progression to accelerated or blastic phase; loss of major cytogenetic response; hematologic resistance; being taken off TKI therapy for any toxicity or death from any cause [21].

HLA-G polymorphism

The entire HLA-G gene was amplified by long-range PCR and sequenced using next-generation sequencing (NGS) with Illumina's Nextera® technology and a 300 bp paired-end read protocol. For statistical comparisons, only allele variations in the HLA-G locus coding region with a frequency higher than 1% were considered: G*01:01 (G*01:01:01, G*01:01:02, G*01:01:03), G*01:03, G*01:04, G*01:05N and G*01:06. Frequencies of HLA-G polymorphisms were compared to those of 1076 healthy subjects from the 1000Genome project [3]. Patients were stratified according to HLA-G haplotypes with higher or lower HLA-G secretor alleles, as reported in previous studies [1]. The

presence of the 14-bp deletion/insertion polymorphism in exon 8 of the 3'UTR of the HLA-G gene was determined as previously described [22]. Patients were subdivided into three groups according to HLA-G 14-bp genotype: homozygotes for the 14-bp insertion (in/in), heterozygotes for the 14-bp deletion/insertion (del/in) and homozygotes for the 14-bp deletion (del/del). The 14-bp polymorphism of the HLA-G gene was analyzed using PCR

HLA-G soluble assay

Each sample of whole blood was collected into commercially available EDTA-treated tubes. Plasma was separated by centrifugation for 10 minutes at 3.000 rpm, properly apportioned into 0.5 ml aliquots and stored at -80°C until use. The BioVendor sHLA-G ELISA (RD194070100R sHLA-G ELISA - EXBIO Praha a.s. BioVendor) immunoassay was used for the quantitative measurement of HLA-G1 and HLA-G5 soluble forms in EDTA-plasma samples. Samples and calibrators were incubated in microplate wells pre-coated with monoclonal anti-sHLA-G antibody. After 16-20 hours incubation and washing, monoclonal anti-human β2-microglobulin antibody labeled with horseradish peroxidase was added to the wells and incubated for 60 minutes with captured sHLA-G. The reaction was stopped by addition of acidic solution; absorbance of the resulting yellow product was measured at 450 nm. The absorbance was proportional to the concentration of sHLA-G. A calibration curve was constructed by plotting the mean absorbance (Y) of calibrators against the known concentration (X) of calibrators in logarithmic scale, using the four-parameter algorithm. Results were reported as concentration of sHLA-G (Units/ml) in samples.
HLA-G samples were collected in the most recent visit, when also bcr-abl was repeated to confirm or not deep MR.

Statistical analysis

The probability of achieving EFS and $MR^{4.5}$ was calculated using the Kaplan-Meier method. The log-rank test was used to compare the 2 groups of patients stratified according to HLA-G haplotypes with high or low soluble HLA-G secretors. HLA-G plasma levels of the 2 groups of patients were compared using the Mann-Whitney test. The frequency distribution of HLA-G allele variants was compared between CML patients and controls. Significant differences were calculated using Fisher's two-sided exact test or Pearson's chi-squared test, as appropriate.

All independent variables studied (Sokal risk, age >45 years, frontline 1st generation TKI vs 2nd generation, numbers of TKI lines, $MR^{4.5}$ achievement by 18 months, HLA-G polymorphism in/in or in/del or del/del, number of HLA-G 01:01:01 or 01:01:02 or 01:01:01 alleles, sHLA-G low, intermediate or high secretion) were included in a binary logistic regression analysis, where ESF status and $MR^{4.5}$ achievement were considered as dependent variables. Variables with a p-value lower than 0.2 in univariate analysis were analysed in a multivariate level with a model=enter procedure, employed to find the best equation. Only p-values ≤ 0.05 were considered to be statistically significant.

Results

Patients and clinical outcomes

The demographic and clinical characteristics of the 68 chronic phase CML patients (median age 63 years, range 27-88) are shown in Table 1. The mean white blood cell (WBC) and platelet (PLT) counts were 28.7 x10^3/uL (range 4.8-360) and 494 x10^3/uL (range 168-910), respectively. Sokal risk was low in 55.8% of the patients and intermediate/high in 44.2%. Sixty patients (88.2%) were treated upfront with imatinib. Ten patients received second-line treatment with nilotinib (14.7%) and 3 patients with dasatinib

(4.4%) for intolerance or resistance. The median follow-up was 107 months (ranges 16-120). Overall, the 8-year EFS and OS were 83.6% and 90%, respectively. The 10-year cumulative incidence of MR^3 and $MR^{4.5}$ was 83.6% and 72.5%, respectively. The multivariate analysis showed that $MR^{4.5}$ achievement was negatively correlated with frontline 1st generation TKI (p=0.018; OR=0.024, 95% C.I.= 0.01-0.53) and HLA-G 01:01:02 allele (p=0.047; OR=0.11, 95% C.I.= 0.11-0.97). EFS was significantly associated with higher sHLA-G in univariate (p=0.05) but not multivariate analysis.

HLA-G polymorphism and clinical outcomes in CML

The following allele frequencies were observed in our cohort of CML patients: G*01:01, 63.2%; (of which: G*01:01:01, 41.2%; G*01:01:02, 14%; G*01:01:03, 11.8%), G*01:03, 10.3%; G*01:04, 18.4%; G*01:05N, 2.2%; G*01:06, 5.9%. No significant differences were found in comparison to the frequencies of the 1000Genome project [3]. (Table 2). Only the G*01:03 allele was over-represented in comparison with the referring population (10,29% vs 4,46; p=0.001) (Table 2).

Eighteen patients homozygous for the G*01:01:01 or G*01:01:02 allele showed significantly lower EFS (6 events) compared to patients with other allelic combinations (4 events) (62.3% vs 90.0%; p=0.02) (Figure 1). In addition, 10 patients carrying the allelic variant G*01:01:03 had significantly higher rates of $MR^{4.5}$ (100% vs 65%), with earlier achievement of deep molecular response (median of 8 vs 58 months, p=0.001). Twenty-three patients (33.8%) were homozygous for the 14-bp deletion polymorphism (del/del) and 14 (20.6%) for the 14-bp insertion polymorphism (in/in); the remaining 31 patients (45.6%) were heterozygous (in/del). No significant association was found between the 14-bp polymorphism and EFS or $MR^{4.5}$ achievement.

HLA-G polymorphism and soluble HLA-G levels in CML

In line with previous studies [1], patients carrying the G*01:01:01 or G*01:01:02 alleles were grouped as higher sHLA-G secretors, while those carrying G*01:01:03 were classified as lower secretors. The mean value of soluble HLA-G was significantly higher in 18 CML patients homozygous for G*01:01:01 or G*01:01:02 compared to 10 patients carrying G*01:01:03 (109.2±39.5 vs 39.9±8.8 units/ml; p=0.03) (figure 2). No significant differences were found for sHLA-G levels in relation to the 14bp polymorphism nor to TKI used for treatment.

Discussion

Despite the relatively small number of patients, our study is the first to suggest that HLA-G expression may influence the clinical outcome of CML. Previous reports have shown that HLA-G can impair tumor immunoediting. This is an important dynamic host protection process that includes three essential phases: elimination, equilibrium and escape [4]. In the elimination phase, HLA-G may reduce the ability of host innate and adaptive immunity to clear cancer cells in the early stages of tumor growth; in the equilibrium phase, HLA-G expression on cancer cells may tip the balance towards the final phase in which tumor cells are less susceptible to immune effector cells and escape host immune defense mechanisms [1].

In hematological malignancies, investigation into the clinical relevance of HLA-G protein expression and soluble HLA-G has yielded contradictory results [9-17]. Increased levels of both cell surface and soluble HLA-G protein expression have been observed in acute and chronic B-cell lymphoproliferative disorders, but the impact of these findings on prognosis and tumor progression remains to be established. Different to what has been observed in solid tumors, B and T malignant cells express receptors recognized by HLA-G

molecules. Hence, the role played by HLA-G in oncohematologic diseases is probably more complex.

CML represents an ideal model in which to evaluate the role of the innate immune system in pathogenesis and response to treatment. Experience in hematopoietic stem cell transplantation (HSCT) and treatment of CML recurrence after HSCT by donor lymphocyte infusion (DLI) has considerably contributed to our understanding of the role of cytotoxic lymphocytes and NK cells in the control of leukemia stem cells and achievement of biological recovery. In a previous report, we found a significant association between achievement of $MR^{4.5}$ and killer immunoglobulin-like receptors (KIRs) expressed on the cell surface of NK cells [20].

HLA-G is a tolerogeneic molecule that is able to exert its action through ILT2 and ILT4 inhibitory receptors on several cells of the innate (NK cells, neutrophils, dendritic cells) and adaptive immune system (B and T lymphocytes). HLA-G/ILT2 interaction has an inhibitory effect on NK cell function through a reduction in interferon-γ secretion by NK cells, down-regulation of chemokine receptors and down-modulation of Stat5 phosphorylation in peripheral blood NK cells [23].

So far, no studies have evaluated the impact of HLA-G molecules on the clinical outcome of CML [24] and very few case reports have addressed HLA-G expression in CML cell surface. The blast cells of 3 and 4 CML patients evaluated within 2 larger cohorts of hematological malignancies yielded negative results for HLA-G expression [25,26]. Conversely, another study found that the proportion of HLA-G expression in leukemic blasts of two out of nine CML patients was 3.8% and 78%, respectively [27].

To the best of our knowledge, also the role of soluble HLA-G molecules has never been evaluated in CML. Expression of soluble HLA-G is associated to variations of the HLA-G gene and, based on the polymorphic coding region, “high” and “low” secretor

HLA-G alleles have been identified [28]. In our study, we found that HLA-G alleles associated to higher secretion of sHLA-G (homozygosity for G*01:01:01 or G*01:01:02) would seem to determine lower EFS and TFR through a stronger inhibitory effect on the immune system in favor of tumor escape mechanisms (Figure 2). Interestingly, we observed in these patients elevated levels of soluble HLA-G (G1 and G5 molecules) (Figure 2). Conversely, the G*01:01:03 allele with a low secretor profile was found in patients who achieved earlier $MR^{4.5}$, suggesting increased cooperation of the immune system in CML cell clearance. We also found a significant association between the G*01:01:03 allele and onset of CML, but this association will need to be confirmed in larger cohorts of patients. Overall, our data seem to suggest that HLA-G polymorphism contributes to immune response in CML and could be suggested as a useful immune biomarker for the identification of CML patients who are likely to present deeper cytogenetic and molecular response to TKI therapy or could benefit from a program of TKI discontinuation. Additionally, modulation of HLA-G function may represent an innovative target for immune-mediated treatment approaches to CML, as antibodies anti-HLA-G or anti-ILT2. Indeed, HLA-G/ILT2/ILT4 has a broader effect on the immune system compared to other T cell inhibitory pathways, such as B7/CTLA4 and PD-1/PD-L1, that are currently used in clinical practice [29,30]. Given the retroactive preliminary and exploratory nature of our study, statistical analysis was not adjusted for potential covariates or confounders. Further studies on wider cohort of patients and adjusted for confounding effects, will be required to confirm our findings.

DECLARATIONS

Competing Statement:

The authors have no conflicts of interest to disclose

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Authors contributions: Conception and design: GC, MG, GLN; Collection and assembly of data: MG, MA, RC, SO, BM, EA, SG, OM, MT, SL; Statistical analysis: GC, RC, RL; Manuscript writing: GC, GLN, Final approval of manuscript: GC, MG, MA, RC, SO, BM, EA, SG, OM, MT, RL, SL, CC, GLN

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Tables

Table 1. Clinical and demographic features of 68 patients in chronic phase CML

	Patients with CML N=68	
Age (median, range)	63 (27-88)	
Age at diagnosis (median, range)	54 (24-82)	
Male gender (N°, %)	39	57.4
Months of follow-up (median, range)	107 (16-120)	
Leukocytes x 10^3/uL (mean, range)	28.7 (4.8-360)	
Platelets x 10^3/uL (mean, range)	494 (168-910)	
Intermediate-high Sokal risk (N°, %)	30	44.2
Imatinib first line treatment (N°, %)	60	88.2
Nilotinib first line treatment (N°, %)	8	11.8
Nilotinib second line treatment (N°, %)	10	14.7
Dasatinib second line treatment (N°, %)	3	4.4

Table 2. Frequencies of HLA-G polymorphisms in 68 CML patients and healthy controls.

	Patients with CML 2N=136	Genome project 2N=2152	P value
HLA-G coding region polymorphism frequencies	**(%)**		
G*01:01	63.24	60.87	NS
G*01:03	10.29*	4.46*	0.001*
G*01:04	18.4	17.33	NS
G*01:05N	2.21	3.3	NS
G*01.06	5.9	2.83	NS
G*01:01:01	41.2	40.15	NS
G*01:01:02	14	14.45	NS
G*01:01:03	11.8	6.27	NS
HLA-G 3'untranslated region polymorphism frequencies			
14-bp in/in	20.6	-	NS
14-bp in/del	33.8	-	NS
14-bp del/del	45.6	-	NS

Figures

Figure 1. Achievement of EFS according to HLA-G polymorphism in 68 CML patients. Patients homozygous for the G*01:01:01 or G*01:01:02 allele showed significantly lower EFS compared to patients with other allelic combinations (62.3% vs 90.0%; p=0.02).

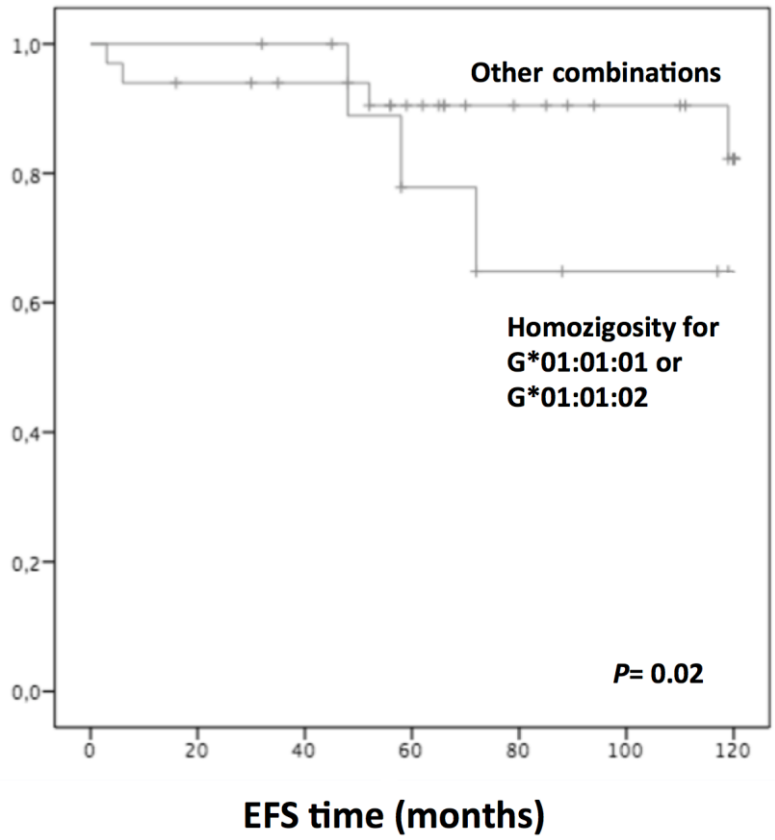

Figure 2. Soluble HLA-G levels in patients with higher secretor (homozygous for G*01:01:01 or G*01:01:02) and lower secretor haplotypes (G*01:01:03); (sHLA-G= 109.2±39.5 vs 39.9±8.8 units/ml; p=0.03).

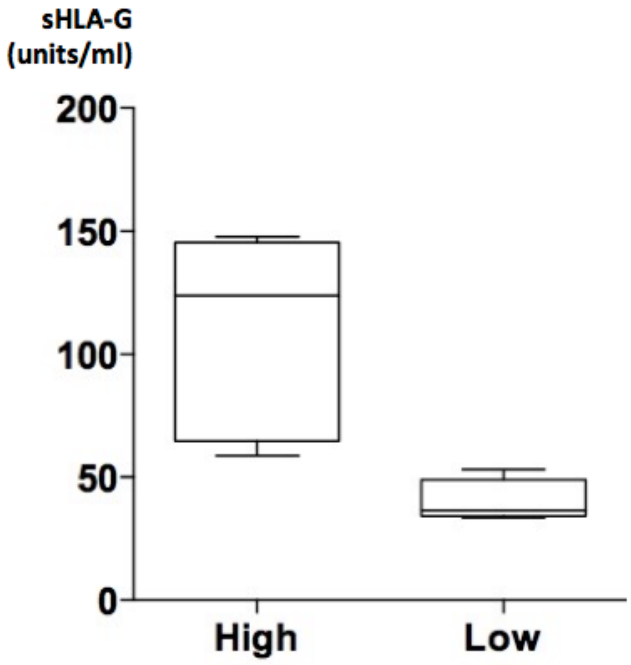

Figure 1

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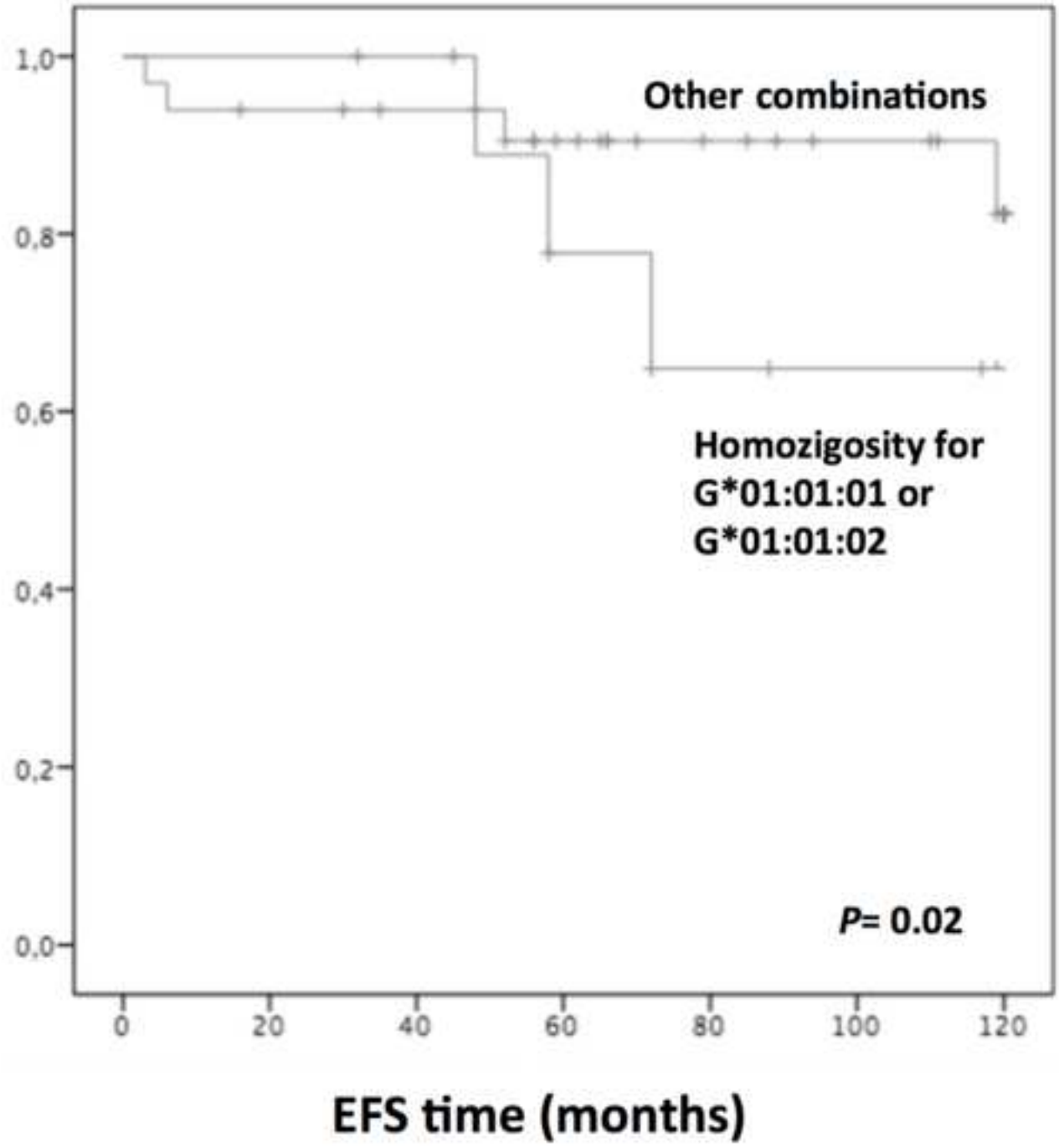

Figure 2

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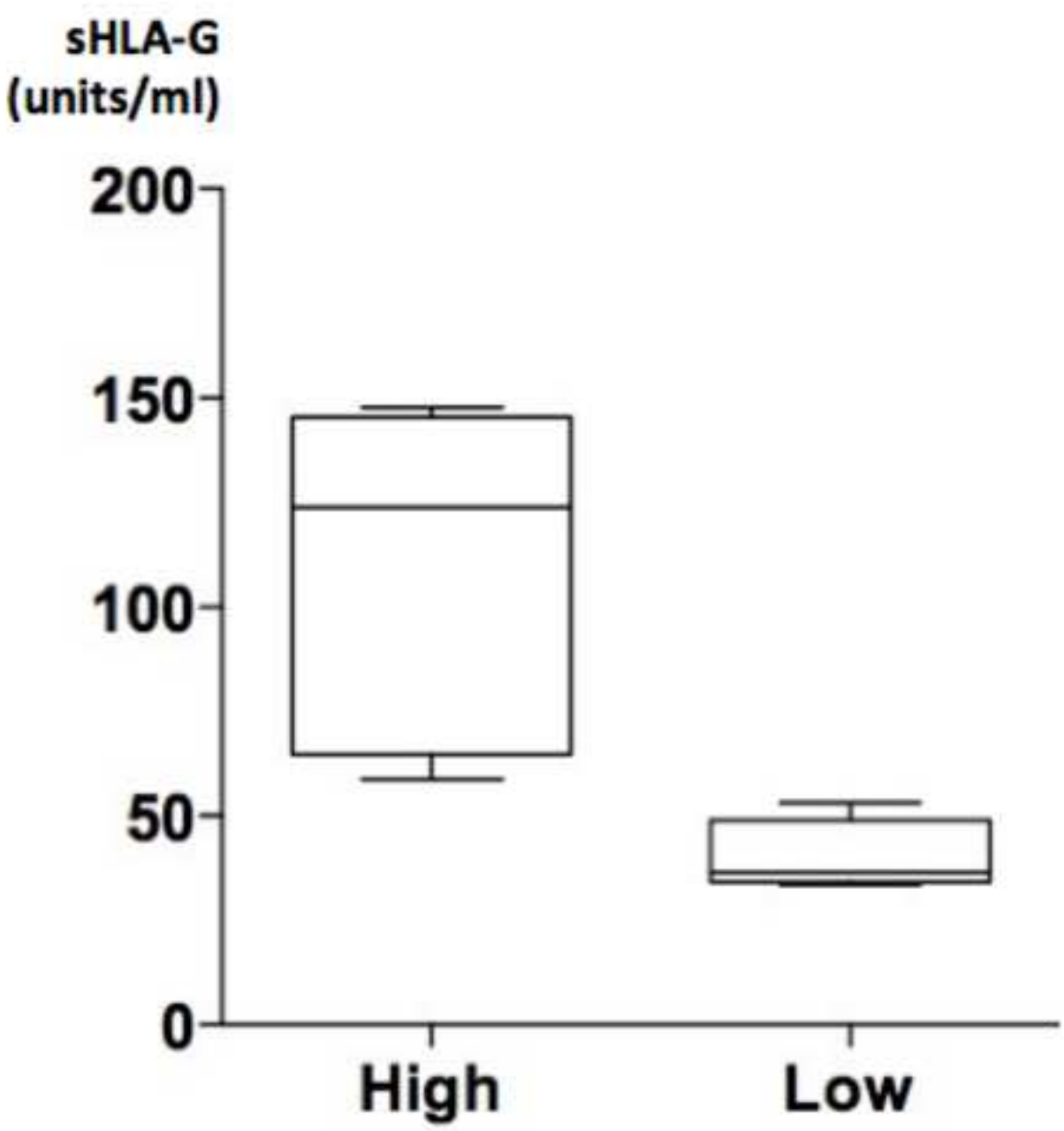

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DECLARATIONS

Competing Statement:

The authors have no conflicts of interest to disclose