# Total Soluble HLA Class I and Soluble HLA-G in Multiple Myeloma and Monoclonal Gammopathy of Undetermined Significance

Xavier Leleu,[1,3,4] Gaëlle Le Friec,[5,6] Thierry Facon,[1] Laurence Amiot,[5] Renée Fauchet,[5] Bernadette Hennache,[2] Valérie Coiteux,[1] Ibrahim Yakoub-Agha,[1] Sylvain Dubucquoi,[2] Hervé Avet-Loiseau,[9] Claire Mathiot,[7] Régis Bataille,[9] and Jean-Yves Mary[8] on behalf of the Intergroupe Francophone du Myélome

**Abstract** Serum $\beta_2$-microglobulin, the light chain of the HLA class I molecular complex, remains one of the best survival prognostic factors in multiple myeloma, but other HLA class I molecules might be of interest in monoclonal gammopathies. In this study, we evaluate total soluble HLA class I (HLA-Is) and soluble HLA-G (HLA-Gs) in 103 patients with newly diagnosed multiple myeloma, 30 patients with monoclonal gammopathy of undetermined significance (MGUS), and 30 healthy subjects, studying their prognostic value in multiple myeloma. In multiple myeloma patients, HLA-Is and HLA-Gs median values were 0.8 μg/mL and 28 ng/mL, respectively. Median HLA-Is concentration was higher in stage II and III multiple myeloma patients than in stage I multiple myeloma, MGUS, and control patients. Median HLA-Gs was significantly lower in healthy controls than in MGUS and multiple myeloma patients. A high level of HLA-Is (≥2.1 μg/mL) was predictive of short survival ($P = 0.017$). For each given level of $\beta_2$-microglobulin, the relative risk of death was higher for patients with HLA-Is ≥ 2.1 μg/mL than in patients with a lower level ($P = 0.047$). HLA-Gs, a marker of monoclonal gammopathy, was of no prognostic value, but the addition of HLA-Is to $\beta_2$-microglobulin produced an efficient prognostic score ($P < 0.0001$). HLA-Is is a new marker of multiple myeloma tumor load and provides additional survival prognostic information to $\beta_2$-microglobulin.

In multiple myeloma, $\beta_2$-microglobulin is widely recognized as one of the most useful survival prognostic factors (1). Among several staging systems using $\beta_2$-microglobulin, the $\beta_2$-microglobulin and albumin combination is of particular interest (2) and has been recently validated in the context of the International Staging System (3). Other staging systems using more sophisticated variables such as cytogenetics could also emerge in the future (4).

The serum $\beta_2$-microglobulin is the light chain of the HLA class I molecular complex, expressed on the surface of the majority of nucleated cells (5), and corresponds to the serologic soluble HLA heavy chain – free $\beta_2$-microglobulin (6). Because serum $\beta_2$-microglobulin is an important feature in multiple myeloma, other HLA class I molecules may be of interest in monoclonal gammopathies. Soluble HLA class I molecules (HLA-Is) as well as the main soluble HLA-G isoform (HLA-Gs) are known to have immunosuppressive properties (7). This immunomodulating role is partially explained by their capacity to protect target cells from natural killer and T cytolysis and to trigger apoptosis in activated T $CD8^+$ cells (8, 9). The cell origin and pathway of HLA-Is secretion in multiple myeloma remain unclear. Several soluble $\beta_2$-microglobulin – associated HLA class I heavy chains are generated by different mechanisms: the full-length lipid-associated protein (44000 Da) generated by exosomal shedding, the truncated protein (39000 Da) resulting from alternative splicing, and the proteolytic form (35000 Da) released by metalloproteinase-mediated cleavage (10). However, as several metalloproteinases are produced by human myeloma cells and seem to play a critical role in the pathogenesis of multiple myeloma (11, 12), this suggests a critical proteolytic pathway involvement.

A recent study suggested that the soluble $\beta_2$-microglobulin – free HLA class I heavy chain expression could be a disease marker in multiple myeloma (13). In a tumoral process, HLA-G expression could constitute a mechanism of tumor progression, and HLA-Gs expression has been shown in various cancer types and in some lymphoproliferative disorders (14 – 17).

In this study, we evaluate the levels of soluble associated $\beta_2$-microglobulin/HLA-G5 (HLA-Gs) and total HLA-Is in patients with multiple myeloma, monoclonal gammopathy of undetermined significance (MGUS), and in age-matched

**Authors' Affiliations:** [1]Service d'Hématologie, [2]Laboratoire de Biochimie, Lille, France; [3]Department of Medical Oncology, Harvard Medical School; [4]Bing Program for Waldenström's Macroglobulinemia and Jerome Lipper Multiple Myeloma Center, Dana-Farber Cancer Institute, Boston, Massachusetts; [5]Laboratoire d'Hématologie et de Biologie des Cellules Sanguines, Rennes, France; [6]Washington University School of Medicine, St. Louis, Missouri; [7]Laboratoire d'Hématologie, Institut Curie; [8]Institut National de la Sante et de la Recherche Medicale U717, Hôpital St. Louis, Université Paris VII, Paris, France; and [9]Laboratoire d'Hématologie, Nantes, France
Received 3/1/05; revised 6/28/05; accepted 7/27/05.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
**Note:** X. Leleu and G. Le Friec contributed equally to this work.
There is no conflict of interest.
**Requests for reprints:** Thierry Facon, Service des Maladies du Sang, Hôpital Huriez, Centre Hospitalier Régional Universitaire de Lille, rue Michel Polonovski, 59037 Lille, France. Phone: 33-3-20-44-57-13; Fax: 33-3-20-44-47-08; E-mail: t-facon@chru-lille.fr.

doi:10.1158/1078-0432.CCR-05-0456

healthy subjects. We also studied their potential prognostic value on survival in multiple myeloma, especially in relation to $\beta_2$-microglobulin.

## Patients and Methods

***Patient selection.*** One hundred and three patients with multiple myeloma diagnosed between 1989 and 2000 in the center of Lille according the criteria of the International Myeloma Working Group (18) and staged according to the Durie/Salmon staging system (19) were included in the study. The patient characteristics are given in Table 1. The patients were always treated after serum collection according to the Intergroupe Francais du Myelome protocols, receiving either conventional chemotherapy ($n$ = 53) or high-dose therapy with autologous peripheral blood stem cell support ($n$ = 38). Twelve patients remained untreated at the date of point. For treated patients, the median time between serum collection and treatment was 0.3 month (interquartile range, 0.0-2.9).

Thirty patients with MGUS diagnosed according to the International Myeloma Working Group (ref. 18; 17 females and 13 males) and 30 age-matched healthy subjects were also studied. MGUS patients had a median age of 62 years (interquartile range, 56-68) and their median bone marrow plasmacytosis was 3% (interquartile range, 2-5). At the date of point, no patient had progressed to multiple myeloma.

***Laboratory variables.*** The biological evaluations were done in Lille for usual variables and Rennes for soluble HLA class molecules. Chromosome 13 deletion ($\Delta$13) was determined using fluorescence *in situ* hybridization analysis (20). HLA-Gs and HLA-Is concentrations were measured using a specific sandwich ELISA. Microtiter plates (Corning Costar, Brumath, France) were coated in PBS at pH 7.4 with MEM-G/9 (10 μg/mL), a monoclonal antibody detecting specifically HLA-Gs molecules associated with $\beta_2$-microglobulin (Exbio, Prague, Czech Republic) or with W6/32 (5 μg/mL), monoclonal antibody recognizing a monomorphic determinant of $\beta_2$-microglobulin associated to any of the HLA class I heavy chains (Harlan Sera-Lab Ltd., Leicestershire, England). After three washes in PBS containing 0.05% Tween 20, plates were saturated with 250 μL of PBS containing 2% bovine serum albumin for 30 minutes at room temperature. One hundred microliters of pure sera for HLA-Gs or diluted at 1/50 in PBS for HLA-Is were added to each well and tested in triplicate. After incubation for 1 hour at room temperature, plates were washed thrice in PBS with 0.05% Tween 20. Anti-$\beta_2$-microglobulin/horseradish peroxidase (DAKO, Trappes, France; 100 μL) was added to each well and plates were incubated for 1 hour at room temperature. Plates were washed thrice and then incubated with the substrate (orthophenylenediamine dihydrochloride, DAKO) for 30 minutes in dark. After addition of $H_2SO_4$ (1 N), absorbencies were measured at 490 nm. Standard curves were done using serial dilutions of either calibrated supernatant of B-lymphoblastoid cell line LCL-721.221 (American Type Culture Collection, Rockville, MD) transfected with HLA-Gs cDNA (LCL721.221-G5), kindly provided by V. Rebmann (Essen, Germany). Soluble HLA-B7s molecules (Sangstat, Fremont, CA) were used as a standard to calculate the total amount of sHLA-I antigens. Thus, the concentrations of HLA-Gs and HLA-Is were determined from the value of absorbance according to the standard curves.

***Statistical analysis.*** The distributions of HLA-Is and HLA-Gs levels among the different groups (controls, MGUS, and multiple myeloma by stage) were characterized through median and interquartile range. Distribution comparison between these samples were done through Mann-Whitney or Kruskal-Wallis tests, according to the following strategy: normal versus MGUS, homogeneity among multiple myeloma patients according to stage, MGUS versus multiple myeloma in case of homogeneity among multiple myeloma patients across stage, otherwise MGUS versus stage I multiple myeloma, homogeneity between stage II and stage III multiple myeloma, and stage I versus stage II or III multiple myeloma if homogeneity among stage II and stage III multiple myeloma patients, stage I versus stage II multiple myeloma otherwise. Correlations between HLA-Is and HLA-Gs levels with the level of other biological variables involved in multiple myeloma disease were tested through Spearman correlation coefficient, within the multiple myeloma sample, if homogeneous according to the methodology described above.

Date of point was May 1, 2004. Overall survival and progression-free survival were evaluated through Kaplan-Meier estimates as a function of

**Table 1.** Patient characteristics at blood collection ($n$ = 103, except if indicated)

| | % Patients | Median (interquartile range) |
|---|---|---|
| Age (y) | | 64 (56-72) |
| >70 | 29 | |
| Male sex | 44 | |
| Stage | | |
| I | 38 | |
| II | 19 | |
| III | 43 | |
| Isotype | | |
| IgG | 64 | |
| IgA | 27 | |
| Only light chain | 6 | |
| Other | 3 | |
| Kappa light chain ($n$ = 102) | 65 | |
| HLA-Is (μg/mL) | | 0.8 (0.5-2.1) |
| ≥2.1 | 25 | |
| HLA-Gs (ng/mL) | | 28 (17-39) |
| Treatment | | |
| No ($n$ = 12) | 12 | |
| Yes ($n$ = 91) | | |
| Conventional | 58 | |
| Intensive | 42 | |
| Time to treatment (mo), $n$ = 91 | | 0.3 (0.0-2.9) |
| Bone marrow plasmacytosis (%) | | 23 (12-45) |
| <25 | 53 | |
| 25-50 | 27 | |
| ≥50 | 20 | |
| Chromosome 13 deletion ($n$ = 70) | 31 | |
| Hemoglobin (g/d) | | 10.8 (9.6-12.7) |
| ≤12 | 71 | |
| Creatinine (mg/L) | | 10 (8-12) |
| ≥15 | 14 | |
| Albumin (g/L) | | 39.5 (36.5-43.0) |
| ≤35 | 17 | |
| Calcium (mg/L) | | 94 (91-99) |
| $\beta_2$-microglobulin (mg/L) | | 2.8 (2.0-3.9) |
| <2.5 | 42 | |
| 2.5-4.0 | 37 | |
| ≥4.0 | 21 | |
| LDH (IU/L), $n$ = 102 | | 252 (207-304) |
| ≥220 | 66 | |
| CRP (mg/L) | | 3.0 (2.0-7.0) |
| ≥6 | 29 | |

Abbreviations: LDH, low-density hydrocarbon; CRP, C-reactive protein.

time from serum collection and after first conventional or intensive treatment, respectively (21). In addition to HLA-Is and HLA-Gs, all variables described in Table 1 were examined for their prognostic value on overall survival. In these prognostic analyses, continuous variables were categorized based on 25th, 50th, and 75th percentiles, as described previously (22). Usual limits (as 2.5, 4.0, and 6.0 mg/L for $\beta_2$-microglobulin) were also tested. For univariate analyses, overall survival curves were compared through log-rank test (23). The estimate of the relative risk of death and its 95% confidence interval was estimated through proportional hazards model (24). Then, the ability of each variable, HLA-Is and HLA-Gs, to increase overall survival predictive ability of the $\beta_2$-microglobulin was tested through backward stepwise proportional hazards model using likelihood ratio test (25). The subsequent prognostic models were simplified by using the same procedure, as the one described for univariate analysis of continuous prognostic variable, leading to final models with three or four categories only. No further improvement by adding another factor in the model was looked for according to the sample size and the number of deaths in our sample. The influence of patient treatment on these prognostic models was investigated by testing their prognostic ability through a proportional hazards model including conventional and intensive treatments as time-dependent covariates (26).

The respective qualities of our final models and of some well-known previously published models (2, 3, 22, 27, 28) applied to our sample were described through Kaplan-Meier estimates of survival curves and quantified through the value in −2log (likelihood), the lower value corresponding to the best fit of data for a fixed number of prognostic categories and the same sample size. All analyses were done with the SPSS software (29).

## Results

***HLA-Is and HLA-Gs serum levels and correlations to other variables.*** As described in Table 2A, HLA-Is concentration varied across stages in multiple myeloma patients ($P = 0.02$) but not between stages II and III ($P = 0.88$). A statistically significant difference was observed between stage I and stage II to III multiple myeloma ($P = 0.005$), with higher values for the latter group. In contrast, no significant differences were observed between controls and MGUS and between MGUS and stage I multiple myeloma. Regarding HLA-Gs (Table 2B), there was no variation across stages in multiple myeloma patients and between MGUS and multiple myeloma patients. The only difference was evidenced between controls and MGUS ($P = 0.03$) or multiple myeloma ($P < 0.001$). No variation of HLA-Is and HLA-Gs with age could be evidenced among multiple myeloma patients. No correlation was observed between HLA-Is and HLA-Gs. HLA-Is showed a minor correlation with $\beta_2$-microglobulin in stage I ($r = 0.44$, $P = 0.005$), stage II to III multiple myeloma ($r = 0.27$, $P = 0.03$), and in the entire multiple myeloma population ($r = 0.29$, $P = 0.003$). In contrast, HLA-Gs did not correlate with $\beta_2$-microglobulin in multiple myeloma regardless of disease stage. Minor correlations of HLA-Is with hemoglobin ($r = -0.23$, $P = 0.02$) or bone marrow plasmacytosis ($r = 0.23$, $P = 0.02$) were observed but only globally in multiple myeloma patients, as a consequence of their variations across stages. Both HLA markers did not correlate to creatinine and to chromosome 13 deletion.

***Survival analyses.*** In multiple myeloma, the median follow-up time was 73.2 ± 5.4 months and the median survival time was 51.4 ± 5.8 months (65 deaths). Among the 91 treated multiple myeloma patients, 64 progressions were observed and the median progression-free survival time was 18.8 ± 1.7 months. Variables significantly affecting overall survival in univariate analysis are presented in Table 3. A high level of HLA-Is (≥2.1 μg/mL) was predictive of a short overall survival, as shown on Fig. 1. In contrast, HLA-Gs level had no effect on overall survival. There was a trend in patients with increasing $\beta_2$-microglobulin level but not in those with high HLA-Is level to shorter progression-free survival ($P = 0.08$ and $P = 0.55$, respectively).

**Table 2.** Values of total HLA-Is and HLA-Gs among the different groups

| | Controls | MGUS | Stage I MM | Stage II MM | Stage III MM |
|---|---|---|---|---|---|
| A. HLA-Is (μg/mL) | | | | | |
| Mean ± SD | 1.04 ± 0.92 | 0.66 ± 0.32 | 0.93 ± 0.83 | 1.87 ± 1.70 | 1.79 ± 1.70 |
| | | | | 1.81 ±1.69 | |
| Median | 0.70 | 0.52 | 0.73 | 1.34 | 1.03 |
| | | | | 1.06 | |
| Interquartile range | 0.42-1.15 | 0.39-0.89 | 0.39-1.03 | 0.48-2.68 | 0.58-2.79 |
| | | | | 0.55-2.72 | |
| No. patients ($n$ = 64) | 30 | 30 | 39 | 20 | 44 |
| | | | | | |
| B. HLA-Gs (ng/mL) | | | | 64 | |
| Mean ± SD | 18.6 ± 9.3 | 28.7 ± 17.6 | 32.2 ± 23.4 | 28.2 ± 13.3 | 32.0 ± 19.6 |
| | | | | 31.3 ± 20.0 | |
| Median | 19.5 | 28.7 | 26.7 | 30.9 | 26.9 |
| | | | | 28.0 | |
| Interquartile range | 9.8-25.2 | 11.6-41.6 | 16.0-39.5 | 14.8-38.4 | 18.1-43.6 |
| | | | | 17.0-39.1 | |
| No. patients ($n$ = 103) | 30 | 30 | 39 | 20 | 44 |
| | | | | 103 | |

NOTE: Stages I, II, and III are according to the Durie and Salmon classification.
Abbreviation: MM, multiple myeloma.

In the multivariate analysis, $\beta_2$-microglobulin ($P < 0.001$) and HLA-Is ($P = 0.047$) were independent prognostic factors affecting overall survival. Relative risk of death (95% confidence interval) was 2.1 (1.2-3.7) for patients with $\beta_2$-microglobulin between 2.5 and 4.0 mg/L and 3.6 (1.9-6.9) for patients with $\beta_2$-microglobulin $\geq$ 4.0 mg/L compared with patients with $\beta_2$-microglobulin < 2.5 mg/L and 1.8 (1.0-3.2) for patients with HLA-Is $\geq$ 2.1 μg/mL compared with patients with HLA-Is < 2.1 μg/mL. Based on these results, a prognostic model with four categories was derived (Table 4; Fig. 2*A*): 1 point was given for a $\beta_2$-microglobulin level between 2.5 and 4.0 mg/L; 2 points if $\beta_2$-microglobulin level was ≥4.0 mg/L; and 1 point if HLA-Is level was ≥2.1 μg/mL, the risk of death increasing with the score or total of points. This classification seemed clearly different from the one obtained with $\beta_2$-microglobulin alone (Table 3; Fig. 2*B*). The $\beta_2$-microglobulin/HLA-Is model still strongly affected overall survival when taking into account conventional and intensive treatments as time-dependent covariates ($P = 0.009$). This prognostic classification, as well as the one based on $\beta_2$-microglobulin alone, had only a borderline significance on progression-free survival ($P = 0.15$ and 0.08, respectively).

***Comparison between prognostic models.*** We did the comparison of the $\beta_2$-microglobulin/HLA-Is model with some previously published models. Our previous model using $\beta_2$-microglobulin, fluorescence *in situ* hybridization Δ13, and IgA isotype (22) was superior to the $\beta_2$-microglobulin/HLA-Is model, the latter was superior to the Southwest Oncology Group $\beta_2$-microglobulin/albumin model (28), −2log (likelihood) being estimated to be 279.700, 286.813, and 292.362, respectively. To compare our model with previous models

**Table 3.** Significant prognostic factors for survival (univariate Cox analysis)

| | O/N* | Relative risk of death estimate (95% confidence interval) | Survival time (mo, median ± SE) | *P* † |
|---|---|---|---|---|
| $\beta_2$-microglobulin (mg/L) | | | | 0.0001 |
| <2.5 | 20/43 | 1‡ | 78.6 ± 7.1 | |
| 2.5-4.0 | 27/38 | 2.1 (1.2-3.7) | 47.3 ± 5.3 | |
| ≥4.0 | 18/22 | 3.8 (2.0-7.3) | 18.2 ± 7.2 | |
| Stage | | | | 0.001 |
| I | 21/39 | 1‡ | 70.9 ± 7.0 | |
| II/III | 44/64 | 2.4 (1.4-4.2) | 32.0 ± 5.4 | |
| Hemoglobin (g/d) | | | | 0.002 |
| >12 | 14/30 | 1‡ | 78.6 ± 7.0 | |
| ≤12 | 51/73 | 2.5 (1.4-4.6) | 36.6 ± 6.4 | |
| Bone marrow plasmacytosis (%) | | | | 0.002 |
| <25 | 30/55 | 1‡ | 69.6 ± 10.1 | |
| ≥25 | 35/48 | 2.2 (1.3-3.5) | 31.9 ± 4.8 | |
| Albumin (g/L) | | | | 0.004 |
| >35 | 51/86 | 1‡ | 57.8 ± 8.1 | |
| ≤35 | 14/17 | 2.3 (1.3-4.2) | 18.2 ± 8.5 | |
| LDH (IU/L) | | | | 0.011 |
| <220 | 17/35 | 1‡ | 95.2 ± 29.9 | |
| ≥220 | 47/67 | 2.1 (1.2-3.7) | 45.9 ± 8.4 | |
| Age (y) | | | | 0.013 |
| ≤70 | 41/73 | 1‡ | 68.4 ± 7.3 | |
| >70 | 24/30 | 1.9 (1.1-3.1) | 32.0 ± 8.0 | |
| HLA-Is (μg/mL) | | | | 0.017 |
| <2.1 | 47/77 | 1‡ | 63.6 ± 8.9 | |
| ≥2.1 | 18/26 | 2.0 (1.1-3.5) | 31.3 ± 6.4 | |
| Chromosome 13 deletion | | | | 0.029 |
| No | 26/48 | 1‡ | 63.6 ± 8.1 | |
| Yes | 16/22 | 2.0 (1.1-3.7) | 25.6 ± 4.0 | |
| Treatment§ | | | | |
| No | 5/12 | 1‡ | 79.7 ± 1.3 | |
| Intensive | 18/38 | 4.3 (1.5-11.7) | 68.4 ± 8.7 | 0.002 |
| Conventional | 42/53 | 9.1 (3.6-23.2) | 32.0 ± 5.9 | <0.0001 |

Abbreviation: LDH, low-density hydrocarbon.
*O/N = number of dead patients/total number of patients.
†Significance level.
‡Baseline group.
§Analysis of treatment effect was done through time-dependent covariates (see text) and compared with the no-treatment group.

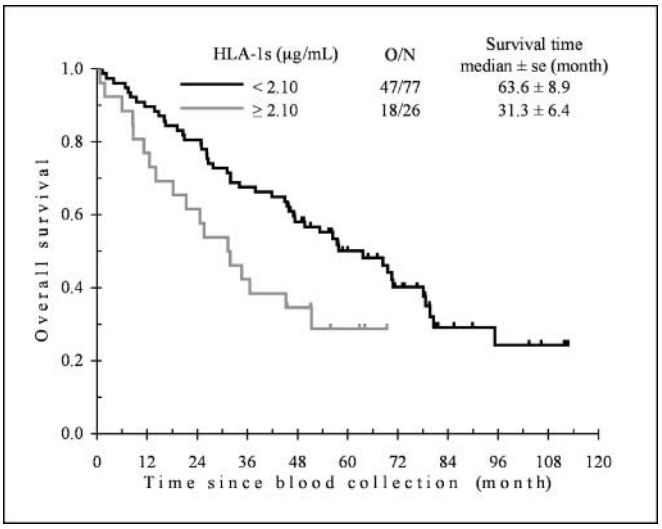


**Fig. 1.** Overall survival in 103 multiple myeloma patients according to total HLA-Is serum level. O/N = number of deaths/total number of patients.

with only three categories, the two intermediate categories of the $\beta_2$-microglobulin/HLA-Is model were mixed (Table 4). In these conditions, the combination of $\beta_2$-microglobulin and $\Delta$13 was the most effective model followed by the combination of $\beta_2$-microglobulin and HLA-Is with $-2$log (likelihood) estimated on 70 patients to be 294.023 and 296.721, respectively. The combination of $\beta_2$-microglobulin and C-reactive protein (27) provided inferior models (data not shown). The $\beta_2$-microglobulin and albumin model which constitutes the basis of the simplified International Staging System (3) was also inferior in this series of patients, $-2$log (likelihood) being estimated on 103 patients to be 516.638 compared with 507.451 for the $\beta_2$-microglobulin/HLA-Is model.

## Discussion

This study suggests that HLA-Is is a new marker of multiple myeloma tumor load, with higher levels found in Durie Salmon stage II/III multiple myeloma compared with stage I multiple myeloma, MGUS, and healthy controls. HLA-Gs was higher in all kinds of monoclonal gammopathies compared with controls. Correlations between these markers and usual multiple myeloma variables were poor, except between HLA-Is and $\beta_2$-microglobulin. In multiple myeloma, a high level of HLA-Is but not of HLA-Gs was associated with a poor survival. The combination of $\beta_2$-microglobulin and HLA-Is provided a powerful prognostic model, a high level of HLA-Is identifying patients with a more severe prognosis, compared with a low level, for any level of serum $\beta_2$-microglobulin. This prognostic model seemed more efficient than other previously published models, except the combination of $\beta_2$-microglobulin and chromosome 13 deletion (22).

To our knowledge, the main soluble HLA-G isoform, a nonclassic MHC class I molecule, have not been studied in multiple myeloma, but it has been investigated in other lymphoproliferative disorders: chronic lymphocytic leukemia and B and T non-Hodgkin lymphoma (14–17). In all these

**Table 4.** Survival prognostic models in 103 multiple myeloma patients according to $\beta_2$-microglobulin and total HLA-Is

| Variables | | O/N* | Relative risk of death | | Survival time (mo, median ± SE) | *P*† |
|---|---|---|---|---|---|---|
| $\beta_2$-microglobulin (mg/L) | HLA-Is (μg/mL) | | Estimate | 95% confidence interval | | |
| Four-category model | | | | | | |
| <2.5 | <2.1 | 16/35 | 1‡ | | 80.5 ± 9.4 | <0.0001 |
| <2.5 | ≥2.1 | 25/37 | 2.0 | 1.1-3.8 | 53.3 ± 5.8 | |
| 2.5-4.0 | <2.1 | | | | | |
| 2.5-4.0 | ≥2.1 | 16/22 | 3.2 | 1.5-6.4 | 27.8 ± 3.7 | |
| ≥4.0 | <2.1 | | | | | |
| ≥4.0 | ≥2.1 | 8/9 | 7.7 | 3.2-18.8 | 14.1 ± 4.3 | |
| Three-category model | | | | | | |
| <2.5 | <2.1 | 16/35 | 1‡ | | 80.5 ± 9.4 | <0.0001 |
| <2.5 | ≥2.1 | | | | | |
| 2.5-4.0 | — | 41/59 | 2.3 | 1.3-4.2 | 47.0 ± 8.5 | |
| ≥4.0 | <2.1 | | | | | |
| ≥4.0 | ≥2.1 | 8/9 | 7.7 | 3.2-18.8 | 14.1 ± 4.3 | |

*O/N: number of dead patients/total number of patients.
†Significance level.
‡Baseline group.

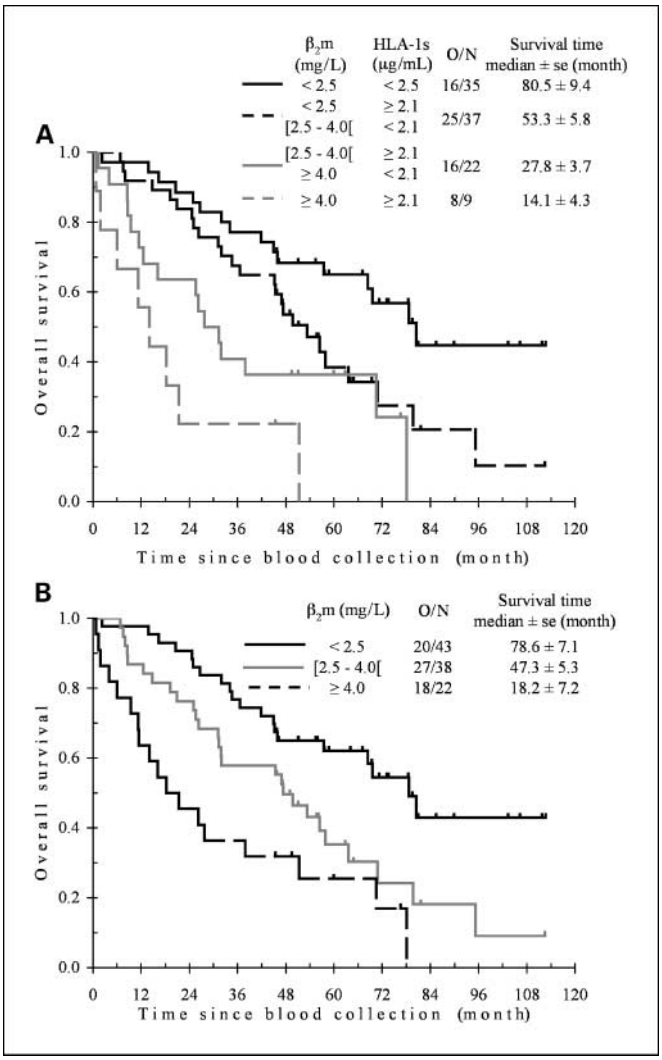


**Fig. 2.** Overall survival according to serum levels of (*A*) $\beta_2$-microglobulin ($\beta_2m$) and total HLA-Is and (*B*) $\beta_2$-microglobulin alone. O/N = number of deaths/total number of patients.

cases, the HLA-G molecules were at low density on the tumoral cell surface contrasting with the frequent detection of the soluble isoform. Approximately 60% of B- and T-lymphoid malignancies have significantly increased HLA-Gs levels compared with healthy subjects with mean levels equal to 49 and 47 ng/mL in chronic lymphocytic leukemia and B non-Hodgkin lymphoma, respectively (17). A study reported an increased level of HLA-G expression at the cell surface of T- and B-primary cutaneous lymphoma, but they did not consider the soluble isoform (30). These differences between cutaneous lymphoma and their nodal or leukemic counterparts could be partially explained by differences regarding the microenvironment and especially the cytokines involved (17).

The serologic $\beta_2$-microglobulin-free HLA class I heavy chain (FHC) has been recently studied (13). The total soluble HLA class I complex, a couple of $\beta_2$-microglobulin and the HLA class I heavy chain, seems more stable than the $\beta_2$-microglobulin-free HLA class I heavy chain (31). FHC levels were significantly higher in multiple myeloma compared with MGUS and controls but did not vary across multiple myeloma stages (13). Similar minor correlations were observed between HLA-Is or FHC and $\beta_2$-microglobulin ($r$ = 0.29 or 0.31, respectively) and bone marrow plasmacytosis ($r$ = 0.29 and 0.36, respectively). HLA molecules levels, total soluble or FHC, were not correlated to creatinine ($r$ = 0.09 and 0.15, respectively). This result was expected as the HLA-I heavy chain is heavier than the $\beta_2$-microglobulin molecule and is more susceptible to catabolism rather than renal filtration. The importance of finding a new marker independent of creatinine in multiple myeloma has been stressed because $\beta_2$-microglobulin is influenced by renal insufficiency and is normal in about 10% of patients (6, 32). No real prognostic factor analysis was done in the study reported by Perosa et al. (13). In our study, the 2.1 μg/L threshold, the upper limit of the inter-quartile range, was derived from overall survival prognostic analysis. We did not do a systematic research of the most effective limit as provided by ROC curve analysis, because it is known to provide limit highly dependent on the studied sample. For every $\beta_2$-microglobulin level, <2.5 mg/L, between 2.5 and 4 mg/L, and ≥4.0 mg/L, an HLA-Is level of ≥2.1 μg/L reclassified patients in a worse prognostic group, concerning one third of patients within each level of $\beta_2$-microglobulin.

The HLA-Is/$\beta_2$-microglobulin model was shown to be more effective than previously proposed models, except the $\Delta13/\beta_2$-microglobulin model (22). Of note, all the models including $\beta_2$-microglobulin and albumin (2, 3, 28) seemed much less effective than the $\beta_2$-microglobulin/HLA-Is model. This needs to be confirmed in other studies because the comparison between these models was done on a sample that was optimal for the HLA-Is/$\beta_2$-microglobulin model, because it was used to construct this model. Furthermore, it should be valuable to test on a sufficiently large sample the prognostic ability of the combination HLA-Is/$\Delta13/\beta_2$-microglobulin. Anyway, the HLA-Is/$\beta_2$-microglobulin model seemed an effective alternative to the $\Delta13/\beta_2$-microglobulin model for investigators lacking cytogenetics.

HLA-Gs was of no survival prognostic value in multiple myeloma, contrarily to HLA-Is. The reason for the discrepancy observed by others that described a prognostic role of HLA-Gs in other B-lymphoid malignancies (14–17) underlined the particularity of the multiple myeloma pathogenesis. The reasons why HLA-Is seemed of prognostic value in multiple myeloma remain unclear. Its prognostic role could be related to its biological activities, especially its immunosuppressive properties. On the other hand, our HLA-Is ELISA evaluation, measuring a couple of $\beta_2$-microglobulin and the HLA class I heavy chain, could be a way to measure the complementary part of $\beta_2$-microglobulin not quantified by the dosage of $\beta_2$-microglobulin alone (free $\beta_2$-microglobulin).

In conclusion, in our study, HLA-Gs had no survival prognostic effect in multiple myeloma but was a marker of clonality. In contrast, HLA-Is gave strong additional survival prognostic information to $\beta_2$-microglobulin alone, leading to a powerful prognostic score. This new prognostic classification needed to be confirmed in other patient cohorts.

## Acknowledgments

We thank Allen Ho for assistance in the preparation of the article and Sabine Ranwez, Myriam Cambié, Jacqueline Regnault, and Jean Luc Faucompré for excellent technical assistance.

## References


**1.** San Miguel J, Garcia-Sanz R. Multiple myeloma: differential diagnosis and prognosis. In: Gahrton G, Durie B, Samson D, editors. Multiple myeloma and related disorders. London: Arnold Publishers; 2004. p. 179–99.

**2.** Bataille R, Durie BG, Grenier J, Sany J. Prognostic factors and staging in multiple myeloma: a reappraisal. J Clin Oncol 1986;4:80–7.

**3.** Durie BG, Kyle RA, Belch A, et al. Myeloma management guidelines: a consensus report from the Scientific Advisors of the International Myeloma Foundation. Hematol J 2003;4:379–98.

**4.** Drach J, Ackermann J, Seidl S, Kaufmann A. Molecular biology and cytogenetics. In: Gahrton G, Durie B, Samson D, editors. Multiple myeloma and related disorders. London: Arnold Publishers; 2004. p. 46–57.

**5.** Cresswell P, Springer T, Strominger JL, Turner MJ, Grey HM, Kubo RT. Immunological identity of the small subunit of HL-A antigens and $\beta_2$-microglobulin and its turnover on the cell membrane. Proc Natl Acad Sci U S A 1974;71:2123–7.

**6.** Schardijn GH, Statius van Eps LW. $\beta_2$-Microglobulin: its significance in the evaluation of renal function. Kidney Int 1987;32:635–41.

**7.** Chumbley G, King A, Robertson K, Holmes N, Loke YW. Resistance of HLA-G and HLA-A2 transfectants to lysis by decidual NK cells. Cell Immunol 1994;155:312–22.

**8.** Contini P, Ghio M, Merlo A, et al. Soluble HLA class I/CD8 ligation triggers apoptosis in EBV-specific $CD8^+$ cytotoxic T lymphocytes by Fas/Fas-ligand interaction. Hum Immunol 2000;61:1347–51.

**9.** Rouas-Freiss N, Marchal RE, Kirszenbaum M, Dausset J, Carosella ED. The $\alpha$1 domain of HLA-G1 and HLA-G2 inhibits cytotoxicity induced by natural killer cells: is HLA-G the public ligand for natural killer cell inhibitory receptors? Proc Natl Acad Sci U S A 1997;94:5249–54.

**10.** DeVito-Haynes LD, Demaria S, Bushkin Y, Burlingham WJ. The metalloproteinase-mediated pathway is essential for generation of soluble HLA class I proteins by activated cells *in vitro*: proposed mechanism for soluble HLA release in transplant rejection. Hum Immunol 1998;59:426–34.

**11.** Kelly T, Borset M, Abe E, Gaddy-Kurten D, Sanderson RD. Matrix metalloproteinases in multiple myeloma. Leuk Lymphoma 2000;37:273–81.

**12.** Vacca A, Ribatti D, Roccaro AM, Ria R, Palermo L, Dammacco F. Bone marrow angiogenesis and plasma cell angiogenic and invasive potential in patients with active multiple myeloma. Acta Haematol 2001;106:162–9.

**13.** Perosa F, Luccarelli G, Prete M, Ferrone S, Dammacco F. Increased serum levels of $\beta_2$m-free HLA class I heavy chain in multiple myeloma. Br J Haematol 1999;106:987–94.

**14.** Mizuno S, Emi N, Kasai M, Ishitani A, Saito H. Aberrant expression of HLA-G antigen in interferon $\gamma$-stimulated acute myelogenous leukaemia. Br J Haematol 2000;111:280–2.

**15.** Polakova K, Krcova M, Kuba D, Russ G. Analysis of HLA-G expression in malignant hematopoetic cells from leukemia patients. Leuk Res 2003;27:643–8.

**16.** Nuckel H, Rebmann V, Durig J, Duhrsen U, Grosse-Wilde H. HLA-G expression is associated with an unfavorable outcome and immunodeficiency in chronic lymphocytic leukemia. Blood 2005;105:1694–8.

**17.** Amiot L, Le Friec G, Sebti Y, et al. HLA-G and lymphoproliferative disorders. Semin Cancer Biol 2003;13:379–85.

**18.** International Myeloma Working Group. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. Br J Haematol 2003;121:749–57.

**19.** Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. Cancer 1975;36:842–54.

**20.** Avet-Loiseau H, Brigaudeau C, Morineau N, et al. High incidence of cryptic translocations involving the Ig heavy chain gene in multiple myeloma, as shown by fluorescence *in situ* hybridization. Genes Chromosomes Cancer 1999;24:9–15.

**21.** Kaplan E. Non parametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457–81.

**22.** Facon T, Avet-Loiseau H, Guillerm G, et al. Chromosome 13 abnormalities identified by FISH analysis and serum $\beta_2$-microglobulin produce a powerful myeloma staging system for patients receiving high-dose therapy. Blood 2001;97:1566–71.

**23.** Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;50:163–70.

**24.** Cox D. Regression models and life-tables. J R Stat Soc 1972;B34:187.

**25.** Cox D, Oakes D. Analysis of survival data. London (England): Chapman & Hall; 1984.

**26.** Altman DG, De Stavola BL. Practical problems in fitting a proportional hazards model to data with updated measurements of the covariates. Stat Med 1994;13:301–41.

**27.** Bataille R, Boccadoro M, Klein B, Durie B, Pileri A. C-reactive protein and $\beta$-2 microglobulin produce a simple and powerful myeloma staging system. Blood 1992;80:733–7.

**28.** Jacobson JL, Hussein MA, Barlogie B, Durie BG, Crowley JJ. A new staging system for multiple myeloma patients based on the Southwest Oncology Group (SWOG) experience. Br J Haematol 2003;122:441–50.

**29.** SPSS/PC+. Statistical package for social science, user's guide. Chicago (IL): SPSS, INC.; 1986.

**30.** Urosevic M, Willers J, Mueller B, Kempf W, Burg G, Dummer R. HLA-G protein up-regulation in primary cutaneous lymphomas is associated with interleukin-10 expression in large cell T-cell lymphomas and indolent B-cell lymphomas. Blood 2002;99:609–17.

**31.** Demaria S, DeVito-Haynes LD, Salter RD, Burlingham WJ, Bushkin Y. Peptide-conformed $\beta_2$m-free class I heavy chains are intermediates in generation of soluble HLA by the membrane-bound metalloproteinase. Hum Immunol 1999;60:1216–26.

**32.** Bataille R, Grenier J, Sany J. Unexpected normal serum $\beta$-microglobulin (B2M) levels in multiple myeloma. Anticancer Res 1987;7:513–5.