Intensity of expression of the CD117 antigen (c-kit) on myelomatous plasma cells

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Abstract

Background: *We have previously shown the expression of c-kit-a receptor with tyrosine kinase activity on plasma cells (PCs) of some multiple myeloma (MM) patients. The aim of the present study was to evaluate the frequency and intensity of c-kit expression on bone marrow (BM) myelomatous PCs.*

Material and methods: *The study group consisted of 109 MM patients (8 at stage I, 22-II, 79-III acc. to D.S. with monoclonal protein: IgG-69, IgA-25, IgM-1, BJ-12 and non-secretory-2). The control group was 10 healthy subjects. Immunophenotyping was performed on freshly collected BM samples using triple staining combination of the CD138/CD117/CD38 monoclonal antibodies analysed by flow cytometry (Cytoron Absolute and FACSCalibur – Becton Dickinson). PCs were indentified acc. to their strong reactivity for CD38, CD138 (syndecan-1) and their typical light scatter distribution. The antigen expression intensity was calculated as relative fluorescence intensity (RFI) and for direct quantitative analysis the QuantiBRITE test (Becton Dickinson) was applied. The mean channels of phycoerythrin fluorescence were defined and antibody bounding capacity (ABC) was then calculated using QuantiCALC software.*

Results: *In 37 patients (=33%) myeloma PCs showed CD117 expression. Out of all nucleated BM cells the mean proportion of PCs with CD117 expression was 25.7±20.3%, median 21%. Values of RFI ranged from 8.4 to 20 in particular MM patients (12.4±3.2 median 11.6) and the number of CD117 binding sites (ABC) on MM plasma cells ranged from 1443 to 6217 (3262±1410, median 3109). A correlation was found between RFI and ABC values (r=0.87). In 72 patients myeloma cells did not express CD117 and mean proportion of all BM cells with CD117 expression was 3±2%, median 2.7%. Normal PCs did not express CD117. In BM of healthy persons the mean proportion of CD117+ cells was 2.4±0.7, median 1.8%. Morphological analysis of MGG stained BM aspirate smears revealed that the percentage of BM PCs in MM patients with PCs positivity for CD117 was 36±24%, median 35%, while a corresponding value in MM patients with PCs negative for CD117 was 41±23%, median 38%. In patients with CD117$^+$ myeloma a positive correlation was found between proportion of CD117$^+$ bone marrow cells and percentage of PCs in BM smears (r=0.95). No differences were seen in occurrence of CD117 expression depending on stage of disease and monoclonal protein isotype.*

Conclusions: *In one third of MM patients CD117 antigen could be considered as a „tumour associated marker" and it may be of value for the identification of the malignant clone in minimal residual disease. The intensity of c-kit expression on PCs varies among particular c-kit positive MM patients and the differences in expression level may be as big as many times. It may be rationale to consider usefulness of therapy with tyrosine kinase inhibitors in the management of MM patients with c-kit positive plasma cell proliferation.*

Key words: *CD117, c-Kit, plasma cells, multiple myeloma.*

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Introduction

C-kit (CD117), a product of c-kit protooncogene, is a 145 kD transmembrane protein, with activity of class III receptor tyrosine kinase. Expression of c-kit can be detected by immunostaining with monoclonal antibody clustered under CD117. A ligand for c-kit is stem cell factor (SCF). Binding of c-kit receptor with its ligand leads to dimerization of receptor, tyrosine phosphorylation and activation of signal transduction cascade controlling cell proliferation, adhesion and differentiation [1, 2]. C-kit is functionally important protein involved in normal haematopoiesis, melanogenesis and gametogenesis. C-kit is expressed by a small proportion of normal human bone marrow cells (0.5-4%). Among normal bone marrow cells, CD117 expression has been found in the majority of the $CD34^+$ precursors including progenitors committed to the myeloid, erythroid and megakaryocytic cell lineages. In addition, strong CD117 expression is detected in bone marrow mast cells as well as in a small subset of NK cells displaying strong reactivity for CD56, and in early T-cell precursors [3-5].

C-kit expression has been demonstrated on a wide range of non-haematopoietic cell types including: melanocytes, vascular endothelial cells, interstitial cells of Cajal, astrocytes, breast glandular epithelial cells as well as in testis, renal tubules and sweat glands [6].

Abnormal activity of c-kit and altered signal transduction coming from this receptor may play a substantial role in oncogenesis process. Increased expression of CD117 was found in some myelo- and lymphoproliferative diseases such as acute myeloblastic leukemia [7, 8], myelodysplastic syndromes, anaplastic lymphomas [9, 10] as well as in some solid tumours including small cell lung carcinoma, glioma, neuroblastoma, germinoma, gastrointestinal stromal tumours (GISTs), some types of breast cancer and melanoma [11-14]. Altered c-kit expression may result from c-kit gene point mutation in enzymatic or regulatory portion of c-kit protein that occurs in early steps of oncogenesis. Such mutation maintains cell stimulation status independently from the receptor ligand suggesting autocrine pathway of activation of the receptor cytoplasmic part [15]. However, in tumours in which no mutations of c-kit gene were found an increase c-kit expression may be a result of receptor stimulation by its ligand. In this context, multiple myeloma is an interesting model. Multiple myeloma is a B-lineage neoplasm characterized by the accumulation in the bone marrow of a clonal population of B-cells in the last stage of differentiation-plasma cells. A special role of SCF (c-kit ligand) in myeloma oncogenesis was revealed by Lemoli et al. [16, 17] who showed the stimulatory effect of SCF on proliferation of fresh myeloma bone marrow cells and some myeloma cell lines with expression of CD117. It was clearly demonstrated that SCF is able to stimulate proliferation of myeloma cell lines in the cultures both with and without presence of cytokines [16-18]. mRNA transcripts for SCF and its receptor, c-kit, have been detected by reverse transcriptase-polymerase chain reaction amplification in myeloma cell lines RPMI 8226, MT3, JJN_3, U266B1, NCI-H929, ARH77 and HS-Sultan [17, 19, 20]. It suggests that c-kit may play a special role in myelomatous plasma cells by influencing their proliferation. We have previously shown the expression of CD117 on plasma cells of some multiple myeloma patients [21, 22].

The recent encouraging therapeutic experience with tyrosine kinase inhibitors in chronic myeloid leukemia and $c\text{-}kit^+$ GISTs [23, 24] prompted interest in application of these drugs in the treatment of other tumours with overexpression of c-kit. It implies, that in selected multiple myeloma patients in whom expression of c-kit on plasma cells is unquestionable increased a targeted therapy with tyrosine kinase inhibitors may be a new, promising treatment option [25]. The aim of the present study was an analysis of the frequency and intensity of c-kit expression on bone marrow myelomatous plasma cells. The results has already been presented at several international hematology meetings and XI Polish Society for Immunology Congress [26, 27].

Material and methods

Patients and controls

The study included 109 newly diagnosed and untreated multiple myeloma patients, hospitalised in the Department of Haematology of the Institute of Haematology and Blood Transfusion in Warsaw.

In each patient the following parameters were estimated at the time of diagnosis: age, sex, complete blood examination, percentage of plasma cells in the bone marrow (at collection of bone marrow for FACS analysis there were performed regular bone marrow smears stained with May-Grünwald-Giemsa method), monoclonal protein isotype (using Beckman Paragon Immunofixation Electrophoresis Kit), urine immunoglobulin/24 hours, serum concentrations of monoclonal protein, calcium, creatinine and β_2-microglobulin (using Beckman ARRAY 360 autoanalyser), complete X-ray skeletal survey, stage of disease according to Durie and Salmon classification [28]. Characteristics of analysed patients are presented in the table 1. The patient's informed consent was obtained prior to performing bone marrow aspiration or biopsy.

The control group consisted of 10 healthy volunteers.

Immunophenotypical analysis of CD117 expression was done by means of flow cytometry using monoclonal antibodies.

Sample preparation and immunofluorescence

Immunophenotyping was performed on fresh bone marrow samples taken at diagnosis. The volume of 2 ml of

bone marrow aspirate was collected into the tubes with heparine concentration – 20 U/ml was diluted with an equal volume of phosphate buffered saline (PBS) and centrifugated at 1300 r.p.m. Supernatant was removed and sample was washed with warm PBS, stirred for 2 min and diluted with aliquot of warm PBS.

Cell surface antigens were evaluated by means of direct immunofluorescence. Rinsed bone marrow samples were inserted in disposal glass tubes and stained with simultaneous triple labeling by using mouse anti-human monoclonal antibodies conjugated with respective fluorochroms: fluorescein isothiocyanate (FITC), phycoerythrin (PE) and phycoerythrin/Cyanin 5 (PE-Cy5). In the sudy monoclonal antibodies against following antigens were used: anti-CD117 (PE) obtained from Becton-Dickinson (USA), anti-CD38 (PE-Cy5) from DAKO (Denmark), anti-CD138 (FITC) obtained from Serotec (UK). Isotype specific FITC, PE-Cy5 and PE-conjugated control antibodies were used as controls.

After addition 10 µl of antibody the sample was incubated at 4°C for 30 min. in the dark. In order to remove red cells after incubation with antibodies a special red cell lysis reagent was added and the tube was incubated in the dark for 10 min at a room temperature. Then the sample was centrifugated at 1300 r.p.m. for 5 min and supernantant was removed. The volume of 2.5 ml of cold PBS was added into each tube, the sample was stirred and centrifugated. The supernatant of PBS was removed from the tube and finally sample was resuspended in 0.5 ml of cold PBS, ready for analysis.

Flow cytometry analysis

Flow cytometry analysis was performed immediately after staining by using flow cytometer Cytoron Absolute ORTHO and FACSCalibur Becton-Dickinson. The data acquisition was done by using softwares: Immunocount II for Cytoron Absolute and CELLQuest for FACSCalibur cytometers. At each time 10000 cells were analysed. At first, the gate according to linear forward angle scatter (FSC) and linear scatter (SSC) was set. On CD38/SSC dot plots the gate around cells with strong expression of CD38 (cells $CD38^{++}$) was drawn. The subject of analysis were plasma cells identified on the basis of strong CD38 expression, expression of CD138 and their typical light scatter distribution. The CD117 antigen expression was analysed on $CD38^{++}$ and $CD138^{+}$ cells. The percentage of positive events in plasma cell subset was calculated and compared to the isotype control The results were presented as a percentage of cells with expression of CD117. The plasma cells were determined as $CD117^{+}$ if at least 20% of cells were labeled with anti-CD117 antibody.

Determination of antigen intensity

The CD117 antigen expression intensity was evaluated using two different and independent methods: indirect and direct. The CD117 expression intensity was quantified by measuring mean values of PE fluorescence.

Table 1. Patients' characteristics at study entry

Total number of cases	109
Gender	
Female	52
Male	57
Age (years); x ±SD; median, range	61.8±10.9; 62, 32-86
Monoclonal protein [number of cases and (%)]	
IgG	69 (63)
IgGκ	48
IgGλ	21
IgA	25 (23)
IgAκ	15
IgAλ	10
IgM	1 (1)
IgMκ	1
Bence Jones	12 (11)
BJκ	4
BJλ	8
Non-secretory	2 (2)
Stage of disease [number of cases and (%)]	
I	8 (7)
IA	8
IB	0
II	22 (20)
IIA	18
IIB	4
III	79 (73)
IIIA	56
IIIB	23
Serum β_{2M} (mg/l) x±SD; median	
– in all patients	5.1±4,8; 3.7
– in patients with normal renal function	3.5±2.6; 2.8
– in patients with renal failure	10.3±6.5; 8.5

In an ***indirect*** quantitative analysis of antigen expression intensity the histograms of CD117 fluorescence and isotype control were plotted, mean fluorescence intensity defined by mean channel peak of fluorescence was determined and Relative Fluorescence Intensities (RFI) were calculated in arbitrary units (AU) according to the equation described by Miwa et al [29, 30]. This formula is based on the values of mean channel of fluorescence of analyzed antigen and that of isotypic control: RFI=10x3.5 mean channel of the sample/number of channels – 10x3.5 mean channel of the control/number of channels.

Fluorescent beads (DAKO fluoresphers) were used for standardization.

For a ***direct*** quantitative analysis the QuantiBRITE test (Becton Dickinson) was applied [31-33]. Briefly, the

analysis Kit (Phycoerythrin Fluorescence Quantitation Kit) contains tubes with lyophilized pellet of beads coated with phycoerythrin in 4 known concentrations. These enable determination of quantitative bounding phycoerythrin linked antibody to a particular cell defined as Antibodies Bound per Cell (ABC). At first, Quantibrite PE tubes were run and 10,000 events was collected. The gate was set around bead singlets and bead bet singlets population was analyzed using a histogram plot of fluorescence (FL2) in linear values; four bead peak concentration of PE fluorescence: low, medLow, medHigh, and high were displayed. Using the same instrument settings for fluorescence and compensation there was made acquisition of cellular assay samples. When running Quantibrite PE at the same instrument settings as the assay the axis showing fluorescence (FL2) can be converted into the number of PE molecules bound per cell. Using known ratios of PE to antibodies one can convert PE molecules per cell to antibodies per cell.

The mean channels of PE fluorescence were defined and antibodies bound per cell were calculated using QuantiCALC software. In order to determine the number of PE molecules adhering to the cell the calibration curves were plotted on the basis of read values of mean fluorescence channel for 4 afore-mentioned various phycoerythrin densities using Quantitative Calibration Software. Mean channel of PE fluorescence for CD117 was determined and the values of ABC were read from calibration curve using QuantiCALC software.

All parameters of cytometer and determination of ABC and RFI were identical for both methods.

Statistical analysis

The different clinical and biological disease characteristics were considered individually, depending on their relationship with the CD117 expression by Mann-Whitney test. For correlation analysis Spearman's correlation coefficient (r) was calculated.

Results

In bone marrow of healthy persons mean proportion of $CD117^+$ cells was 2.4±0.7%, median 1.8%. Normal plasma cells from healthy persons were negative for the CD117 antigen. In 37 patients (=34%) myelomatous plasma cells showed CD117 expression, in 72 (66%) patients did not (Fig. 1, Table 2).

Table 2. Frequency of the CD117 antigen expression in multiple myeloma patients

Bone marrow plasma cells	Number of patients (%)
CD117 positive	37 (34)
CD117 negative	72 (66)
Total	109 (100)

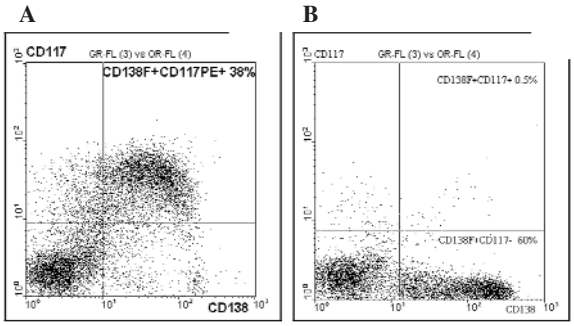

Fig. 1. Cytogram of the bone marrow of 2 myeloma patients. (A) CD117 positive case – a high expression of CD117 on plasma cells; almost all $CD138^+$ cells show coexpression of CD117. (B) CD117 negative case – lack of CD117 on plasma cells; almost all $CD138^+$ cells do not show expression of CD117

In myeloma CD117 positive out of all nucleated bone marrow cells the mean proportion of plasma cells with CD117 expression was 25.7±20.3%, median 21%. In myeloma CD117 negative the mean proportion of bone marrow CD117 positive cells was 3.0±2.0%; median 2.7% (Table 3).

The results of morphological and immunophenotypic analysis of bone marrow plasma cells in c-kit positive and c-kit negative cases were presented in table 4. The mean proportion of bone marrow plasma cells in patients with c-kit positive plasma cell proliferation was 35.8±24.1%, median 35% and in patients with c-kit negative plasma cell proliferation was 41.1±23.2%, median 38%.

Table 3. Relative number of bone marrow CD117 positive cells in multiple myeloma patients and healthy persons

	Number of cases	Relative number of bone marrow $CD117^+$ cells	
		Mean±SD	Median
Multiple myeloma patients	109		
– with CD117 positive plasma cell proliferation	37	25.7±20.3	21
– with CD117 negative plasma cell proliferation	72	3.0±2.0	2.7
Healthy persons	10	2.4±0.7	1.8

Table 4. Relative number of plasma cells in morphological and immunological myeloma bone marrow analysis

	Morphological analysis % of plasma cells (in May-Grünwald-Giemsa stained smears)		Immunological % of CD117⁺ cells (in FACS analysis)		Correlation coefficient
	Mean±SD	Median	Mean±SD	Median	
CD117 positive multiple myeloma	35.8±24.1	35	25.7±20.3	21	r=0.95
CD117 negative multiple myeloma	41.1±23.2	38	3.0±2.0	2.7	

In patients with CD117 positive myeloma a correlation was found between proportion of CD117⁺ cells in FACS bone marrow analysis and percentage of plasma cells in bone marrow smears stained with May-Grünwald-Giemsa (r=+0.95).

In myeloma patients with CD117 positive plasma cell proliferation the majority of CD138⁺ and CD38⁺⁺ bone marrow cells coexpressed CD117 antigen independently on the bone marrow tumour infiltration (Fig. 2, 3, 4), but in some CD117⁺ myelomas expression of this antigen was weak but exceeding accepted in this paper threshold of positivity (20%) (Fig. 5).

The results of the analysis of CD117 antigen expression intensity on plasma cells of 11 multiple myeloma patients were presented in the Table 5. Determinations were done by using the method enabling measurement of the number of antigen molecules on the cell defined as Antibodies

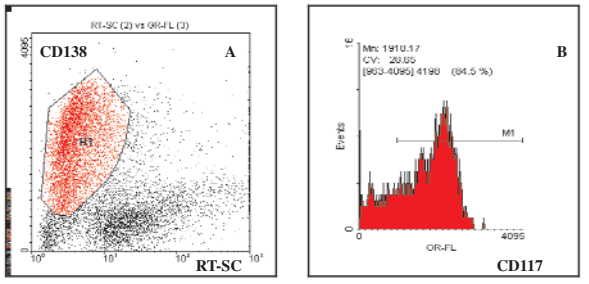

Fig. 2. CD117 expression on CD138 positive cells in bone marrow of multiple myeloma patient. (A) Cytogram: CD138⁺ cells (R1 gate contains 50% of bone marrow white cells, RT-SC-cell granularity). (B) Histogram: 84.5% of CD138 positive cells are simultaneously CD117 positive. Mn- mean fluorescence channel, 4095 – number of channels

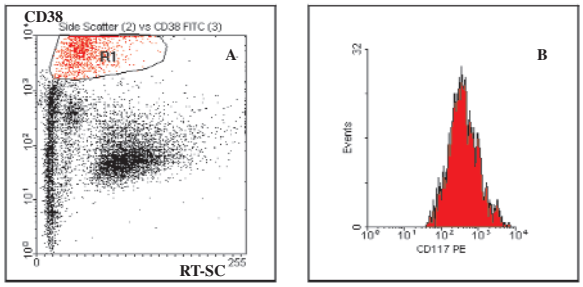

Fig. 4. CD117 expression on CD38⁺⁺ positive cells in bone marrow of multiple myeloma patient. (A) Cytogram: CD38⁺⁺ cells (R1 gate contains 13% of bone marrow white cells, RT-SC-cell granularity, FACS Calibur BD – 255 fluorescence channels). (B) Histogram: 100% CD38⁺⁺ positive cells are simultaneously CD117 positive, PE-Phycoerythrin orange fluorescence

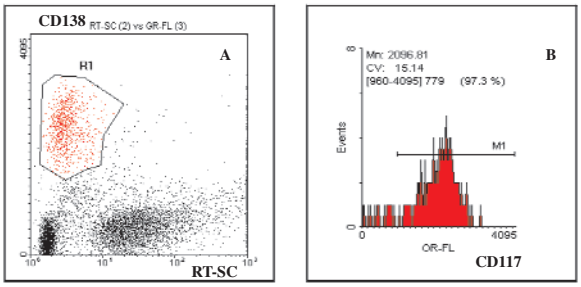

Fig. 3. CD117 expression on CD138 positive cells in bone marrow of multiple myeloma patient. (A) Cytogram: CD138⁺ cells (R1 gate contains 15% of bone marrow white cells). (B) Histogram: 97.3% CD138 positive cells are simultaneously CD117 positive. Mn-mean fluorescence channel, 4095 – number of channels

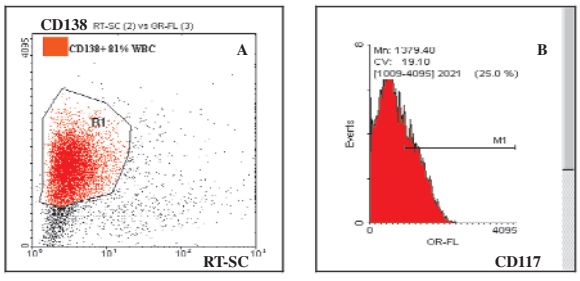

Fig. 5. A weak CD117 expression on CD138 positive cells in bone marrow of multiple myeloma patient. (A) Cytogram: CD138⁺ cells – R1 gate contains 81% of bone marrow white cells. (B) Histogram: 25% of CD138 positive cells are simultaneously CD117 positive, low Mn. Mn- mean fluorescence channel, 4095- number of channels

Table 5. The results of determination of the CD117 antigen expression intensity on plasma cells of MM patients using two methods: ABC and RFI

	Values of expression intensity determinations in particular patients										
Method/cases	1	2	3	4	5	6	7	8	9	10	11
RFI	20.2	12.4	11.6	8.4	10.0	9.8	11.4	15.1	13.8	13.1	10.6
ABC	4779	3109	2783	1714	3260	1443	2021	6217	3855	4057	2644

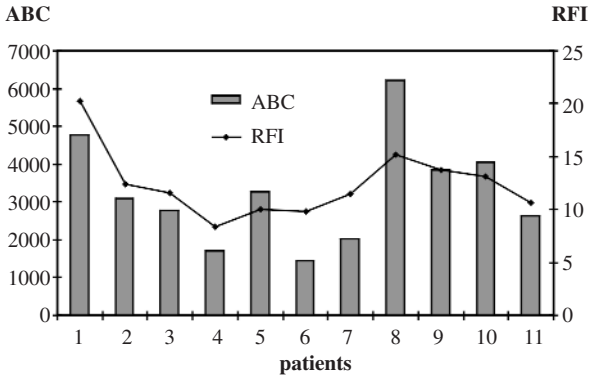

Fig. 6. Relative Fluorescence Intensity (RFI) and Antibody Biniding Capacity (ABC) of c-Kit receptor (CD117) on the plasma cells in multiple myeloma patients

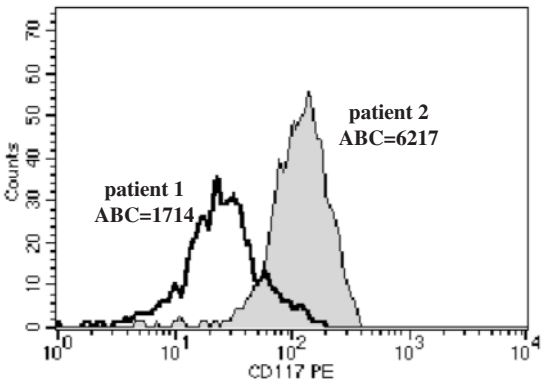

Fig. 7. Histogram of c-Kit receptor (CD117) fluorescence intensity on plasma cells in multiple myeloma patients, PE-Phycoerythrin; X axis – the fluorescence intensity expressed on a logarithmic scale, Y axis-c-Kit receptor-positive cells. ABC – Antibody Binding Capacity (Antibodies bound per cell)

Bound per Cell (ABC) and the method of calculation of Relative Fluorescence Intensity (RFI).

CD117 expression intensity estimated by using both indices (RFI and ABC) showed heterogeneity. The RFI values ranged from 8,4 to 20 (mean: 12.4±3.2; median 11.6) while the ABC values from 1443 to 6217 (mean: 3262±1410; median 3109). Low ABC values corresponded to low values of RFI determinations and high values of ABC – to high RFI indices. A correlation coefficient between the CD117 expression intensity values determined by using of both indices was high (r=0.87) (Fig. 6, 7).

Table 6 presents comparison of clinical-laboratory characteristics parameters in both myeloma patients' groups: c-kit positives and c-kit negatives. In the group of c-kit$^+$ positive patients there was found more elderly persons compared to the c-kit- negative group (age medians 66 and 60 years, respectively). In the c-kit$^+$ group compared to that of c-kit$^-$ there was revealed also a higher proportion of patients with less advanced disease (a percentage of patients at disease stage III in the group c-kit$^+$ was 57% while in the c-kit$^-$ amounted 81%) and lower proportion of patients with the presence of lambda light chain (17% *vs* 47%). The differences, however, were not significant statistically.

Discussion

To the most important findings revealed in this study belongs the demonstration of the lack of c-kit expression on normal plasma cells and presence of this antigen expression exclusively on myeloma plasma cells in one third of patients. Importance of performed analysis consists also in applying two independent methods (ABC and RFI) to determine antigen expression intensity. One of them, ABC, is a relatively new method that makes possible a direct determination of the number of analysed antigen molecules (*antigen density*).

Our study on antigen expression intensity showed the heterogeneity of c-kit expression intensity on myeloma plasma cells. The presence of this phenomenon was revealed by using both methods. It reflects the existence of actual heterogeneity of c-kit expression intensity in multiple myeloma and confirms comparability and equivalence of two applied determination methods.

The studies on establishing CD117 expression pattern in multiple myeloma are not numerous and a main research is being done by Spanish authors. The results of those studies [34, 35] in a part devoted specificity of c-kit expression for multiple myeloma and frequency of occurrence of this phenomenon in myeloma patients are concordant with the

Table 6. Clinical and biological disease characteristics in CD117 positive and CD117 negative multiple myeloma patients

	c-Kit positive cases (n=37)	**c-Kit negative cases (n=72)**
Age (years) x±SD; median	64.9±10.0; 66	60.2±11.2; 60
Serum β_{2M} (mg/l) x±SD; median		
– in all patients	4.9±6.0; 2.85	5.2±4.0; 3.9
– in patients with normal renal function	2.9±1.4; 2.6	3.8±2.9; 3.2
– in patients with renal failure	13.6±10.3; 8.4	9.2±4.4; 8.5
Monoclonal protein phenotype		
IgG (% cases)	25 (69)	44 (62)
IgGκ	24	24
IgGλ	1	20
IgA (% cases)	8 (21)	17 (24)
IgAκ	5	10
IgAλ	3	7
IgM (% cases)	0	1 (1)
IgMκ		1
Bence Jones (% cases)	3 (8)	9 (12)
BJ-κ	1	3
BJ-λ	2	6
Non-secretory (% cases)	1 (2)	1 (1)
Kappa ligh chains (% cases)	30 (83)	38 (53)
Lambda light chains (% cases)	6 (17)	33 (47)
Stage of disease		
I (% cases)	4 (11)	4 (5)
IA	4	4
IB	0	0
II (% cases)	12 (32)	10 (14)
IIA	10	8
IIB	2	2
III (% cases)	21 (57)	58 (81)
IIIA	14	42
IIIB	7	16

results of our analysis. However, afore-mentioned authors did not deal largely with the problem of CD117 expression intensity and its heterogeneity but rather focused their attention on the question of plasma cell autofluorescence and its effect on antigen expression [35]. But they did demonstrate that immunophenotyping CD117 makes possible the detection of expression of this antigen at very high cell dilution. They also proved that the sensitivity of immunophenotyping CD117 used as a myeloma marker is similar to the sensitivity of polymerase chain reaction (PCR) in detection of small amount of tumour cells. In their opinion, immunophenotyping compared to PCR analysis is a method less time consuming and enables performance of quantitative estimation of tumour cells. It opens new perspectives for diagnosing minimal residual disease in multiple myeloma.

On the other hand, the association of tyrosine kinase properties of c-kit molecule with its specific expression on myeloma cells creates unusually important clinical implication – a possibility of therapeutical application of that tyrosinase inhibitors in the treatment targeted to deletion of specific pathogenetic disturbance on the molecular level (*molecular targeting*).

Altered activity of kinases, especially tyrosine kinases, in multiple myeloma raises a special interest and hopes also from the clinical point of view. The results both of experimental studies and first clinical trials revealed antiproliferative activity of tyrosine kinase inhibitors in some solid tumours including kidney, lung and gastrointestinal

stromal tumours [12-14, 24]. Moreover, a tyrosine kinase inhibitor – imatinib (STI 571) showed to be an efficient drug in the treatment of chronic myeloid leukemia, even in definitely prognostically poor phases of this disease [23, 36].

In the experiments with myeloma cell cultures there were successfully applied inhibitors of some Janus kinases (JAK2) that block signal transduction at the stage of originating STAT (*Signal Transducer and Activator of Transcription*) proteins [37, 38]. Tyrphostin, an inhibitor of JAK2 tyrosine kinase, diminishes the activity of MAPK (*Mitogen Activated Protein Kinase*) and transcription factors (STAT) activated by phosphorylation leading to stimulation of myeloma cell apoptosis. This is a signal transduction pathway dependent on interleukin-6 and activation of gp 130 – a signal transduction protein. Intervention in this signal transduction pathway may lead to overcoming myeloma cell resistance to dexamethasone and radiotherapy [39, 40].

The question of application of a derivative of phenylaminopyrimidine, STI 571 (imatinib), a tyrosine kinase inhibitor showing its efficacy in the treatment of chronic myeloid leukemia, for the therapy of multiple myeloma remains open.

As it is known, the therapeutic effect of STI 571 in chronic myeloid leukemia results from inhibiting bcr-abl kinase activity. The rationale for application of STI 571 in the treatment of multiple myeloma is based on belonging of c-kit to the same receptor kinase family as bcr-abl, c-abl and platelet derived growth factor receptor (PDGFR), a close receptor homology between PDGFR and c-kit domains as well as a broad enough spectrum of tyrosine kinase inhibitors [36, 41, 42]. Moreover, there was unequivocally demonstrated that STI 571, apart from inhibiting afore- mentioned three tyrosine kinases, inhibits also the activity of c-kit [36, 41]. STI 571 acts as competitive ATP inhibitor and binding of this inhibitor with ATP leads to kinase blocking [43]. Pandiella et al. [44] have evaluated the action of STI 571 (imatinib mesylate) on different MM cell lines. Addition of STI 571 to cultures of MM1S, MM 144, RPMI 8226, and U266 cell lines caused a decrease in the proliferation of these cells by arresting cell cycle progression. STI 571 inhibited the proliferation of MM cells resistant to dexamethasone or melphalan and had an additive effect when combined with dexamethasone. A phase II trial of Gleevec™ (=imatinib) in patients with refractory/relapsed myeloma is going [25].

Studies on the expression of c-kit and its relationship with SCF lead to two important clinical implications. The first is the necessity of determination of this antigen expression individually in each patient prior to the intended treatment with tyrosine kinase inhibitor due to the prevalence of c-kit negative myeloma proliferations. The second implication is the renunciation of SCF administration for cell mobilisation in multiple myeloma because of stimulating properties of this growth factor on myeloma cell proliferation.

Conclusions

In one third of multiple myeloma patients CD117 antigen could be considered as a „tumour associated marker" and it may be of value for the identification of the malignant clone in minimal residual disease as it was first shown by Spanish authors [34]. The intensity of c-kit expression on plasma cells varies among particular c-kit positive multiple myeloma patients and the differences in expression level may be as big as many times. It may be rationale to consider usefulness of therapy with tyrosine kinase inhibitors in the management of myeloma patients with c-kit positive plasma cell proliferation.

References

1. Linnekin D (1999): Early signaling pathways activated by c-KIT in hematopoietic cells. Int J Biochem Cell Biol 31: 1053-1074.
2. Smith MA, Pallister CJ, Smith JG (2001): Stem cell factor: biology and relevance to clinical practice. Acta Haemat 105: 143-150.
3. Ashman LK, Cambareri AC, To L, et al. (1991): Expression of the YB5. B8 antigen (c-kit proto-oncogen product) in normal human bone marrow. Blood 78: 30-37.
4. Ogawa M, Matsuzaki Y, Nishikawa, et al (1991): Expression and function of c-kit in hemopoietic progenitor cells. J Exp Med 174: 63-68.
5. Avraham H, Vannier E, Cowley S, et al. (1992): Effects of the stem cell factor, c-KIT ligand, on human megakaryocytic cells. Blood 79: 365-371.
6. Ashman LK (1999): The biology of stem cell factor and its receptor c-KIT. Int J Biochem Cell Biol 31: 1037-1051.
7. Schwartz S, Heinecke A, Zimmerman M, et al. (1999): Expression of the c-kit receptor (CD117) is a feature of almost all subtypes of de novo acute myeloblastic leukemia (AML) including cytogenetically good-risk AML and lacks prognostic significance. Leuk Lymph 34: 85-94.
8. Bene MC, Bener M, Casasnovas RO, et al. (1998): The reliability and specificity of c-kit for the diagnosis of acute myeloid leukemias and undifferentiated leukemias. Blood 92: 596-599.
9. Pinto A, Gloghini A, Gattei V, et al. (1994): Expression of the c-kit receptor in human lymphomas is restricted to Hodgkin's disease and $CD30^+$ anaplastic large cell lymphomas. Blood 83: 785-792.
10. Bravo P, Agustin BD, Bellas C, et al. (2000): Expression of high amounts of the CD117 molecule in a case of B-cell non Hodgkin's lymphoma carrying the t (14: 18) translocation. Am J Hematol 63: 226-229.
11. Di Paola RS, Kuczyński WI, Ondera K, et al. (1997): Evidence for a functional c-kit receptor in melanoma, breast cancer and lung carcinoma cells. Cancer Gene Therapy 4: 176-182.
12. Krystal GW, Honsawek S, Litz J, Buchdunger E (2000): The selective tyrosine kinase inhibitor STI571 inhibits small lung cell cancer growth. Clin Cancer Res 6: 3319-3326.
13. Naraghi S, Khoshyomn S, De Mattia JA, Vane DW (2000): Receptor tyrosine kinase inhibition suppresses growth of pediatric renal tumor cells in vitro. J Ped Surg 35: 884-890.
14. Wang WL, Healy ME, Sattler M, et al. (2000): Growth inhibition and modulation of kinase pathways of small cell lung cancer cell lines by the novel tyrosine kinase inhibitor STI571. Oncogene 19: 3521-3528.

15. Gari M, Goodeve A, Wilson G, et al (1999): C-KIT proto-oncogene exon 8 inframe deletion plus insertion mutations in acute myeloid leukemia. Br J Hematol 105: 894-900.
16. Lemoli RM, Fortuna A, Grande A et al. (1994): Expression and functional role of c-kit ligand (SCF) in human multiple myeloma cells. Br J Hematol 88: 760-769.
17. Lemoli RM, Fortuna A (1996): C-kit ligand (SCF) in human multiple myeloma cells. Leuk Lymph 20: 457-467.
18. Lyman SD, Jacobsen SEW (1998): C-kit ligand and Flt3 ligand: stem/progenitor cell factors with overlapping yet distinct activities. Blood 91: 1101-1134.
19. Gooding RP, Bybee A, Cooke F, et al. (1999): Phenotypic and molecular analysis of six human cell lines derived from patients with plasma cell dyscrasia. Br J Hematol 106: 669-681.
20. Escribano L, Ocqueteau M, Almeida, et al. (1998): Expression of the c-kit (CD117) molecule in normal and malignant hematopoiesis. Leuk Lymph 30: 459-466.
21. Kraj M, Pogłód R, Kopeć-Szlęzak J, Kruk B (2000): Expression of the CD117 (c-kit) on myelomatous plasma cells. Immunol Lett 73: 123.
22. Pogłód R, Kraj M, Kopeć-Szlęzak J, Kruk B (2001): Expression of the CD117 (c-kit) on myelomatous plasma cells. Proceedings of the „European Federation of Immunological Societies-EFIS 2000" Bologna (Italy). Monduzzi Editore. International Proceedings Division, p. 433-437.
23. Talpaz M, Silver RT, Druker JB, et al. (2002): Imatinib induces durable hematologic and cytogenetic response in patients with accelerated phase of chronic myeloid leukemia: results of a phase 2 study. Blood 99: 1928-1937.
24. Joensuu H, Roberts PJ, Sarlomo-Rikala M, et al. (2001): Effect of the tyrosine inhibitor STI 571 in a patient with a metastatic gastrointestinal, stromal tumor. N Engl J Med 344: 1052-1056.
25. Dispenzieri A, Witzig TE, Lacy MQ, et al. (2002): A phase II trial of Gleevec TM in patients with refractory/relapsed myeloma. Blood 100 (11) 814a, abstract # 3217.
26. Kraj M, Pogłód R, Kopeć-Szlęzak J, et al. (2002): Intensity of expression of the CD117 antigen (c-kit) on myelomatous plasma cells. 1. Hematol J 3 suppl. 1: 323. 2. Centr Eur J Immunol 27 suppl. 1: 56.
27. Kraj M, Pogłód R, Kopeć-Szlęzak J, et al. (2002): Expression of the CD117 antigen (c-kit) on myelomatous plasma cells and its clinical implications. 1. Blood 100 (11Pt2): 367b. 2. Hematol J 4 suppl. 2: 153. 3. Immunol Lett 2003; 87 (1-3): 209.
28. Durie BGM, Salmon SE (1975): A clinical staging system for multiple myeloma: correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 36: 842-854.
29. Miwa H, Mizutani M, Mahmud N, et al. (1998): Biphasic expression of CD4 in acute myelocytic leukemia (AML) cells: AML of monocyte origin and hematopoietic precursor cell origin. Leukemia 12: 443-451.
30. Gratama JW, D'Hautcourt JL, Mandy F, et al. (1998): Flow cytometric guantitation of immunofluorescence intensity: problems and perspectives. European Working Group on Clinical Cell Analysis. Cytometry 33: 166-178.
31. Davis KA, Abrams B, Hoffman RA, Bishop JE (1996): Quantitation and valence of antibodies bound to cells. Cytometry suppl 8: 125 (#AC150).
32. Iyer S, Suni M, Davis K, Maino V (1996): Expression of CD69 on activated T cells using R-phycoerythrin labeled beads. Cytometry suppl. 8: 113 (# AC 78).
33. Iyer S, Bishop J, Abrams B, et al. (1997): QuantiBrite: A New Standard for Fluorescence Quantitation. Becton Dickinson Immunocytometry Systems, San Jose, CA. Whitee Paper.
34. Ocqueteau M, Orfan A, Garcia-Sanz R, et al. (1996): Expression of CD117 (c-kit) on normal and myelomatous plasma cells. Br J Hematol 95: 489-493.
35. Almeida J, Orfao A, Ocqueteau M, et al. (1999): High sensitive immunophenotyping and DNA ploidy studies for the investigation of minimal residual disease in multiple myeloma. Br J Hematol 107: 121-131.
36. Heinrich PC, Griffith DJ, Druker BJ, et al. (2000): Inhibition of c-kit receptor tyrosine kinase activity by STI 571, a selective tyrosine kinase inhibitor. Blood 96: 925-932.
37. De Vos J, Jourdan M, Tare K, et al. (2000): JAK 2 tyrosine kinase inhibitor tyrphostin AG 490 downregulates the mitogen activated protein kinase (MAPK) and signal transducer and activator of transcription (STAT) pathways and induces apoptosis in myeloma cells. Br J Hematol 109: 823-828.
38. Heinrich PC, Behrmann I, Muller-Neuwen G, et al. (1998): Interleukin-6 type cytokine signalling through gp 130/JAK/STAT pathway. Biochem J 334: 297-314.
39. Catlet-Falcone R, Landowski TH, Oshiro MM, et al. (1999): Constitutive activation of Stat-3 signaling confers resistance to apoptosis in U266 myeloma cells. Immunity 10: 105-115.
40. Rawstron AC, Fenton JAL, Ashcroft J, et al. (2000): The interleukin-6 receptor alpha-chain (CD126) is expressed by neoplastic but not normal plasma cells. Blood 96: 3880-3886.
41. Buchdunger E, Cioffi CL, Law N, et al. (2000): Abl-protein-tyrosine kinase inhibitor STI571 inhibits in vitro singal transduction mediated by c-kit and platelet derived growth factor receptors. J Pharm Exp Ther 295: 139-145.
42. Oetzel C, Jonuleit T, Gotz A, et al. (2000): The tyrosine kinase inhibitor CGP 57148 (STI 571) induces apoptosis in BCR-ABL positive cells by down-regulating BCL-X. Clin Cancer Res 6: 1958-1968.
43. Schindler T, Bornmann W, Pellicena P, et al. (2000): Structural mechanism for STI-571 inhibition of abelson tyrosine kinase. Science 289: 1938-1942.
44. Pandiella A, Carvajal-Vergara X, Tabera S, et al. (2003): Imatinib mesylate (STI 571) inhibits multiple myeloma cell proliferation and potentiates the effect of common antimyeloma agents. Br J Hematol 123: 858-868.