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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

Assessment of bone marrow lymphocytic status during tyrosine kinase inhibitor therapy and its relation to therapy response in chronic myeloid leukaemia

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Abstract

Purpose Tyrosine kinase inhibitors (TKIs) used in the treatment of chronic myeloid leukaemia have been reported to induce immunomodulatory effects. We aimed to assess peripheral blood (PB) and bone marrow (BM) lymphocyte status at the diagnosis and during different TKI therapies and correlate it with treatment responses.

Methods BM and PB samples were acquired from 105 first-line TKI-treated patients. Relative number of BM lymphocytes was evaluated from MGG-stained BM aspirates, and immunophenotypic analyses were performed with multicolour flow cytometry.

Results Early 3-month expansion of BM lymphocytes was found during all different TKIs (imatinib $n = 71$, 20 %; dasatinib $n = 25$, 21 %; nilotinib $n = 9$, 22 %; healthy controls $n = 14$, 12 %, $p < 0.0001$). Increased PB lymphocyte count was only observed during dasatinib therapy. The BM lymphocyte expansion was associated with early molecular response; patients with 3-month *BCR–ABL1* <10 % showed higher lymphocyte counts than patients with *BCR–ABL1* >10 % (23 vs. 17 %, $p < 0.05$). Detailed phenotypic analysis showed that BM lymphocyte expansion consisted of various lymphocyte subclasses, but especially the proportion of CD19+ B cells and CD3negCD16/56+ NK cells increased from diagnostic values. During dasatinib treatment, the lymphocyte balance in both BM and PB was shifted more to cytotoxic direction (increased CD8+CD57+ and CD8+HLA-DR+ cells, and low T regulatory cells), whereas no major immunophenotypic differences were observed between imatinib and nilotinib patients.

Conclusions Early BM lymphocytosis occurs with all current first-line TKIs and is associated with better treatment responses. PB and BM immunoprofile during dasatinib treatment markedly differs from both imatinib- and nilotinib-treated patients.

Keywords Bone marrow · Lymphocyte · CML · Tyrosine kinase inhibitor · Therapy response

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Introduction

Chronic myeloid leukaemia (CML) is caused by the BCR–ABL1 oncoprotein leading to uncontrolled proliferation of bone marrow (BM) myeloid progenitor cells. After introduction of the first-generation tyrosine kinase inhibitor (TKI), imatinib, inhibiting BCR–ABL1 kinase activity, the outcome of chronic phase CML patients has significantly improved (Druker et al. 2006). During last few years, second-generation TKIs, nilotinib and dasatinib, have partially replaced the use of imatinib in first-line CML therapy as they have shown to induce faster and deeper molecular responses (Kantarjian et al. 2010; Saglio et al. 2010; Shami and Deininger 2012).

Several studies have reported that in addition to the oncokinase inhibition, TKIs could also have secondary effects on the immune system and lymphocytic behaviour. In vitro most of the TKIs seem to be immunosuppressive (Cwynarski et al. 2004; Dietz et al. 2004; Weichsel et al. 2008; Chen et al. 2008; Kreutzman et al. 2010, 2011), but when used in vivo, no increased rates of opportunistic infections have been observed. On the contrary, it has been shown that TKIs have immunostimulatory effects, which may be useful when considering the current goal of the treatment, cure of the patients. Especially during dasatinib treatment, a significant proportion of patients develop an expansion of large granular lymphocytes (LGLs) in the peripheral blood (PB), and this has been associated with both the better treatment responses and some side effects such as pleural effusions (Mustjoki et al. 2009; Kim et al. 2009; Kreutzman et al. 2011; Powers et al. 2011; Qiu et al. 2014). Dasatinib has also been shown to induce a rapid mobilization and activation of cytotoxic, extravasation-competent lymphocytes in vivo (Mustjoki et al. 2013a). Similar lymphocyte mobilization or expansion in PB has not been observed during imatinib or nilotinib treatment, but previous data have suggested that BM lymphocyte counts increase during imatinib treatment, especially in well-responding patients (Mustjoki et al. 2007). As the development of BM lymphocytosis has not been studied during dasatinib or nilotinib treatment, we aimed to analyse the BM lymphocyte counts systematically in first-line imatinib-, dasatinib-, and nilotinib-treated patients and examine how lymphocyte counts correlate with treatment responses. Furthermore, we performed detailed immunophenotyping of both PB and BM samples to discover the composition of lymphocyte pool during different TKI therapies.

Patients and methods

Study patients

Altogether 105 patients were included in the study. Written informed consents were obtained from the patients prior to sample withdrawal, and the study was approved by the Helsinki University Central Hospital ethics committee. The principles of Helsinki Declaration were followed. Seventy-one patients were treated with imatinib, 25 with dasatinib, and 9 with nilotinib as a first-line therapy. All patients were in chronic phase. A proportion of patients participated in the NordCML006 study (Mustjoki et al. 2013b; Hjorth-Hansen et al. 2014), and BM differential counts belonged to the study protocol. In addition, samples from 14 healthy BM donors were included.

Morphologic evaluation of BM aspirate samples

May-Grunwald–Giemsa (MGG)-stained BM aspirate slides were examined for cellularity and individual cellular proportions. BM samples were taken from patients at the diagnosis and at 3, 6, and 12 months after the TKI therapy starts. Patient identification was unknown for evaluating haematologist as slides were marked by numerical codes and hence analysed blinded to treatment. In addition, control quality rounds were performed between different centres before the start of the study, and >90 % of concordance in white blood cell differential counts was achieved.

From each time point, two BM slides were examined. Cells were counted from three different fields per slide (total of six fields for the two slides). Finally, mean and median percentage values for lymphocytes, blasts, promyelocytes, and basophils were calculated.

Cytogenetic and molecular genetic analysis

BM karyotyping was done with standard G-banding analysis, and 20 metaphases were examined per slide. In some follow-up samples, fluorescence in situ hybridization (FISH) was also performed when clinically indicated.

Molecular genetic analyses to detect the amount of *BCR–ABL1* transcripts were done with real-time quantitative PCR (RQ-PCR) using TaqMan® chemistry. RT reaction conditions and RQ-PCR assays were performed according to the protocol of Europe Against Cancer (EAC) Programme (Beillard et al. 2003) with either ABL or GUS as the reference genes. The BCR–ABL1 values are reported in the international scale (IS) as % values.

The CML risk classification at the diagnosis was evaluated by Sokal and Hasford scoring systems (Sokal et al. 1984; Hasford et al. 1996; Baccarani et al. 2013).

Patients were divided into different groups according to ELN criteria based on their therapy response at 12 months (Baccarani et al. 2013). As the treatment responses are generally very good in the majority of the patients, we intentionally aimed to include additional failure patients to be able to evaluate the lymphocyte status also in this group of patients.

Cell phenotype analysis by FACS

From fresh BM and PB samples, multicolour flow cytometry was performed in one central laboratory (Helsinki University Hospital) using five different antibody panels. In panel 1, the phenotype of T cells was analysed using antibodies against CD3, CD4, CD8, CD45RA, and CD45RO. Panel 2 evaluated the T cell receptor expression on the cell surface using antibodies against CD3, CD8, TCRαβ, and TCRγδ. In panel 3, the proportions of basic lymphocyte subclasses were evaluated using anti-CD45, anti-CD16+56, anti-CD57, and anti-CD19 antibodies. The activation status of T cells was analysed with panel 4 using antibodies against CD3, CD4, CD8, CD62L, HLA-DR, and cyKi-67. Finally, the proportion of regulatory T cells (Tregs) was analysed with panel 5 (antibodies against CD3, CD4, CD25, and Foxp3). All antibodies were purchased from Becton–Dickinson.

Statistical analysis

GraphPad prism 6.0 and SPSS 22 were used for statistical analysis. One-way ANOVA, *T* test, Mann–Whitney *U* test, and Chi-squared test were used when appropriate and p values <0.05 were considered significant.

Results

TKI treatment induces BM lymphocytosis

As expected, the median proportion of lymphocytes in BM aspirates was significantly lower in CML patients at diagnosis when compared to healthy controls (3 vs. 12 %, $p < 0.0001$). However, at 3 months TKI-treated patients had an increased proportion of lymphocytes in the BM aspirates

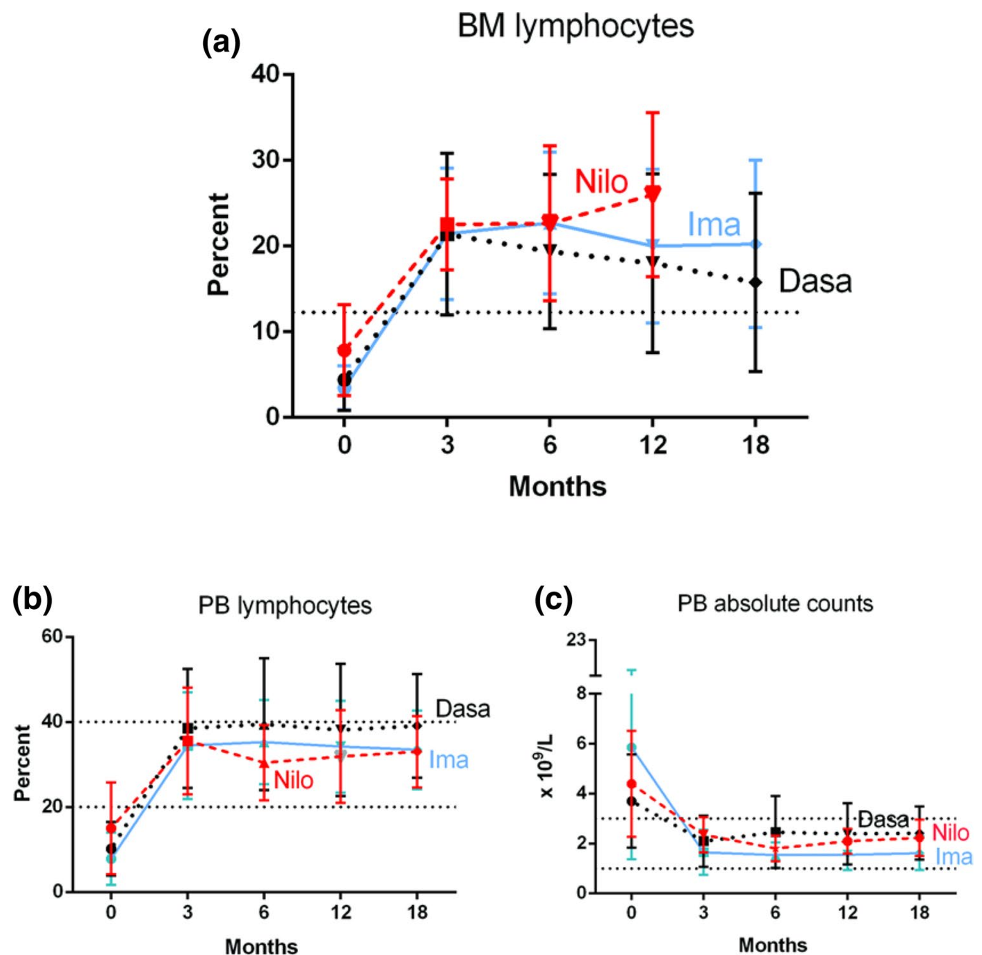


Fig. 1 Increased BM lymphocytosis during TKI therapy. **a** The proportion of lymphocytes in BM samples was analysed at the diagnosis and during TKI therapy (3, 6, 12, and 18 months). The *curves* represent median counts at different time points, and dashed grey line denotes the median lymphocyte count in healthy controls. At 3-month time point, the median lymphocyte percentages in imatinib-, dasatinib-, and nilotinib-treated patients differed significantly from values at the diagnosis and in the healthy controls ($p < 0.01$). **b** The proportions and **c** absolute values of lymphocytes in peripheral blood at the diagnosis and during TKI therapy. The *curves* represent median counts at different time points, and *dashed grey lines* denote the lower and upper normal limit in healthy controls. *Nilo* nilotinib, *dasa* dasatinib, *ima* imatinib

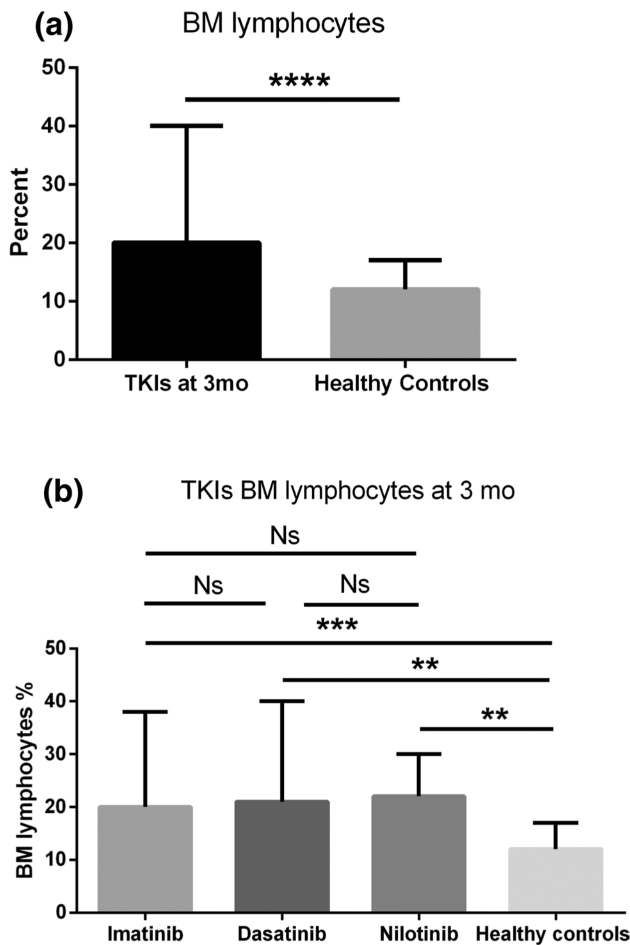


Fig. 2 Increased BM lymphocytosis at the 3-month time point. The median percentage of lymphocytes in all TKI-treated patients (**a**) and separately (**b**) in imatinib-treated ($n = 71$), dasatinib-treated ($n = 25$), and nilotinib-treated patients ($n = 9$) was compared to lymphocyte counts observed in healthy controls. One-way ANOVA test was used for statistical analysis. *NS* not significant; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$

(20 vs. 12 % for healthy controls, $p < 0.0001$; Figs. 1a, 2a; Table 1). Interestingly, patients with low Sokal score had significantly higher proportion of lymphocytes at the 3-month time point (median = 26 %, range 5–38 %) than those with high Sokal score (18 %, 7–33 %) ($p = 0.04$).

When individual drugs were compared, it was evident that the lymphocyte increase occurred both with the first-generation imatinib (20 %, $p < 0.001$) and with the second-generation TKIs dasatinib (21 %, $p < 0.01$) and nilotinib (22 %, $p < 0.01$) (Fig. 1a; Table 1). No significant differences were found when comparing the three different TKIs (Fig. 2b; Table 1). The lymphocyte increase in the BM samples persisted during the later time points (6–18 months), although some regression was observed at 12- and 18-month time points, especially in dasatinib-treated patients (Fig. 1; Table 1, $p > 0.05$). The proportions of blasts, promyelocytes, and basophils were similar in CML patients during TKI therapy as in healthy controls indicting patients being in haematological remission (Table 1).

In PB, the proportion of lymphocytes similarly increased at 3 months (Fig. 1b), but the absolute lymphocyte counts were significantly higher in pre-TKI treatment samples, as there was a general leukocytosis due to BM hyperproliferation (Fig. 1c).

Patients with BM lymphocytosis are more likely to achieve better molecular response

Patients were first categorized into two groups according to their early molecular response at 3 months (*BCR–ABL1* < 10 % and *BCR–ABL1* > 10 %) (Neelakantan et al. 2013). Interestingly, patients who had a favourable early molecular response (*BCR–ABL1* < 10 % at 3 months) had also a significantly higher BM lymphocyte percentage at 3 months (23 vs. 17 %, $p < 0.05$) (Fig. 3).

To study the correlation with later treatment responses, patients were divided into three different groups based on the 12-month molecular response (Baccarani et al. 2013). In the optimal treatment response group, *BCR–ABL1* levels were <0.1 %, in the warning group >0.1–1 %, and in the failure group >1 %. BM lymphocyte percentages in patients with either optimal (19.5 %) or suboptimal/warning (28.5 %) response were significantly higher than in healthy controls (12 %, $p < 0.01$ and $p < 0.0001$, respectively, p ANOVA <0.0001) (Fig. 4). Although failure patients were in haematological remission, the median lymphocyte percentage did not differ significantly from healthy controls, as there was a large variation between individual patients (Fig. 4). Interestingly, the suboptimal group expressed the highest lymphocytic expansion, and it was significantly higher than both optimal and failure (Fig. 4; Table 2). Due to small number of patients treated with second-generation TKIs, we were not able to analyse treatment responses separately with different TKIs.

BM lymphocytosis is accompanied by an increase in NK and B cells

To define the composition of the expanded lymphocyte population in BM, we performed flow cytometry analysis. When all TKIs were analysed together, at the 3-month time point, the proportions of both NK and B cells from the lymphocyte pool were increased when compared to the situation at diagnosis (NK cells: 19.4 vs. 16.8 %, $p < 0.05$; B cells 10.7 vs. 8.6 %, $p < 0.05$). The proportion of CD3+ T cells or CD3+CD16/CD56+ NKT cells did not differ significantly between diagnosis and 3-month time points.

Table 1 BM lymphocytic percentages for the CML patients treated with TKIs and healthy controls at different time points

	Diagnosis[a]	3 months	6 months	12 months	18 months
Imatinib					
Number of cases	64	34	38	39	35
Lymphocyte median % (range)	3 (0–15)	20 (8–38)	22 (4–39)	19 (1–44)	19 (3–45)
Blast median % (range)	2 (0–6)	1 (0–4)	1 (0–4)	1 (0–3)	2 (0–5)
Promyelocyte median % (range)	6 (2–15)	4 (1–8)	3 (0–10)	2 (0–9)	2 (0–10)
Basophil median % (range)	2 (0–16)	1 (0–2)	0 (0–2)	0 (0–3)	0 (0–4)
Dasatinib					
Number of cases	21	18	16	12	13
Lymphocyte median % (range)	3 (1–13)	21 (5–40)	19.5 (6–37)	16.5 (4–39)	13 (1–34)
Blast median % (range)	2 (0–9)	1 (0–5)	1 (0–4)	1 (0–3)	2 (0–3)
Promyelocyte median % (range)	7.5 (0–26)	3 (0–10)	3.5 (1–7)	3 (0–6)	4 (0–9)
Basophil median % (range)	2.5 (0–9)	0 (0–1)	0 (0–1)	0 (0–1)	0 (0–2)
Nilotinib					
Number of cases	7	9	9	5	–
Lymphocyte median % (range)	8 (2–18)	22 (14–30)	25 (10–34)	26 (15–36)	–
Blast Median % (range)	2.5 (1–3)	1 (1–3)	1 (0–2)	1 (0–1)	–
Promyelocyte median % (range)	11 (9–12)	3.5 (2–6)	7 (5–8)	–	–
Basophil median % (range)	2 (0–3)	0 (0–1)	0 (0–0)	0 (0–1)	–
Healthy controls					
Number of cases	14	–	–	–	–
Lymphocyte median % (range)	12 (9–17)	–	–	–	–
Blast median % (range)	0 (0–1)	–	–	–	–
Promyelocyte median % (range)	4 (2–6)	–	–	–	–
Basophil median % (range)	0 (0–1)	–	–	–	–

[a] Or normal values in case of healthy controls

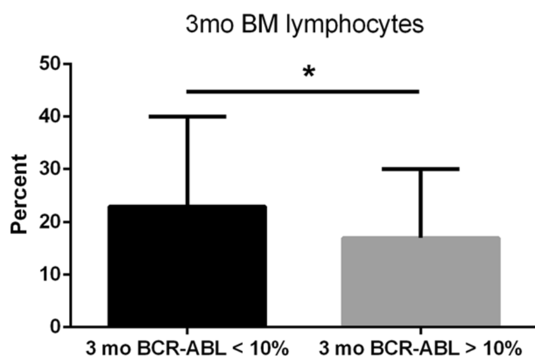


Fig. 3 Early molecular response is associated with the increased BM lymphocyte count. Patients were divided into two different groups based on their BCR–ABL1 levels at 3-month time point (BCR–ABL1 <10 and >10 %). The median lymphocyte counts were compared with *T* test and differed significantly from each other (*$p < 0.05$)

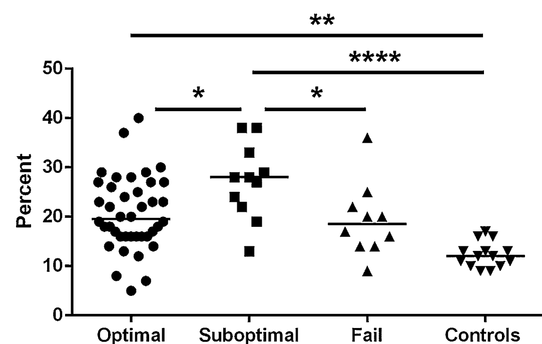


Fig. 4 Three-month lymphocyte counts in different response groups. Patients were divided into three different groups based on their molecular response at 12-month time point (Baccarani et al. 2013). The 3-month BM lymphocyte percentages of individual patients and healthy controls are presented in the figure. The lymphocyte percentages were compared between individual groups with ANOVA. *$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$

Within the CD3+ T cell population, the proportion of CD8+ T cells was decreased at the 3-month time point when compared to the diagnosis (44.5 vs. 48 %, $p < 0.05$). This was concordant with the increased CD4/CD8 ratio at the 3-month time point (0.94 vs. 1.0, $p < 0.01$). No differences were observed in the amount of Tregs.

Dasatinib induces an increase in cytotoxic/killer cell profile and a decrease in Tregs

Although the number of patients treated with second-generation TKIs was small, we still aimed to analyse the flow results separately, as there are very little published in vivo

Table 2 BM lymphocytic percentages for optimal, suboptimal, failure, and control groups

	Optimal	Suboptimal	Fail	Controls
Number of cases	40	11	10	14
Median % (range)	20 (5–40)	28 (13–38)*	19 (9–36)	12 (9–17)**

* p suboptimal versus optimal and fail <0.05

** p controls versus suboptimal <0.0001; p controls versus optimal <0.01

immunological data available from these drugs used in the first-line setting. The analysis was done both at 3- and 6-month time points, but due to larger number of samples available, only 6-month data are presented here. The proportion of CD19+ B cells, CD3-CD16/56+ NK cells, or CD3+ T cells did not differ significantly between individual TKIs, but the proportion of CD3+CD16/56+ NKT-like cells was higher in dasatinib-treated patients (especially in PB, Fig. 5). Similarly, dasatinib-treated patients had significantly more CD8+ T cells in BM (48 and 51 % at 3 and 6 months) when compared to imatinib-treated (41 and 39 %) and nilotinib-treated (43 and 33 %) patients (Fig. 5). The proportion of CD8+ T cells was also increased in PB samples in dasatinib-treated patients at 6-month time point (45 % in dasatinib group compared with 31 % in imatinib and 29 % in nilotinib group).

The proportion of Tregs in the BM was significantly lower in dasatinib-treated patients (3.2 %) than in the imatinib group (5.7 %, $p < 0.05$) (Fig. 5). In nilotinib-treated patients, however, the proportion of Tregs (5 %) was comparable to the imatinib as well as to pre-treatment percentages (5.1 %). Similar trends were also observed in PB, but the differences were not statistically significant.

Dasatinib-treated patients have more CD57+ and HLA-DR+ T cells in BM and PB

More detailed phenotypic analysis showed that CD57 expression was increased in CD3+ T cells in both BM and PB samples of dasatinib-treated patients (Fig. 6). At 6 months 40 % of CD3+ cells in BM were CD57+ in the dasatinib group, compared with 21 and 25 % in the imatinib and nilotinib groups ($p = 0.004$), respectively. The percentages were slightly lower in the PB samples (34, 20, and 30 %, respectively, $p < 0.05$).

Similarly as described earlier, dasatinib-treated patients had less CD62L-expressing CD4+ and CD8+ T cells in both PB and BM when compared to imatinib-treated patients (Rohon et al. 2010) (Table 3). In nilotinib-treated patients, the expression levels of CD62L were similar as in imatinib-treated patients (Table 3).

When HLA-DR expression was analysed separately in CD4+ and CD8+ T cells, imatinib- and nilotinib-treated patients did not differ from each other. However, dasatinib-treated patients had significantly more HLA-DR+ T cells in BM and PB. This was noticed in both the CD4+ (in BM dasatinib 18 % vs. imatinib 7 % and nilotinib 7 %, $p < 0.05$) and CD8+ cell compartments (41 vs. 22 vs. 15 %, respectively, $p < 0.05$) (Fig. 6).

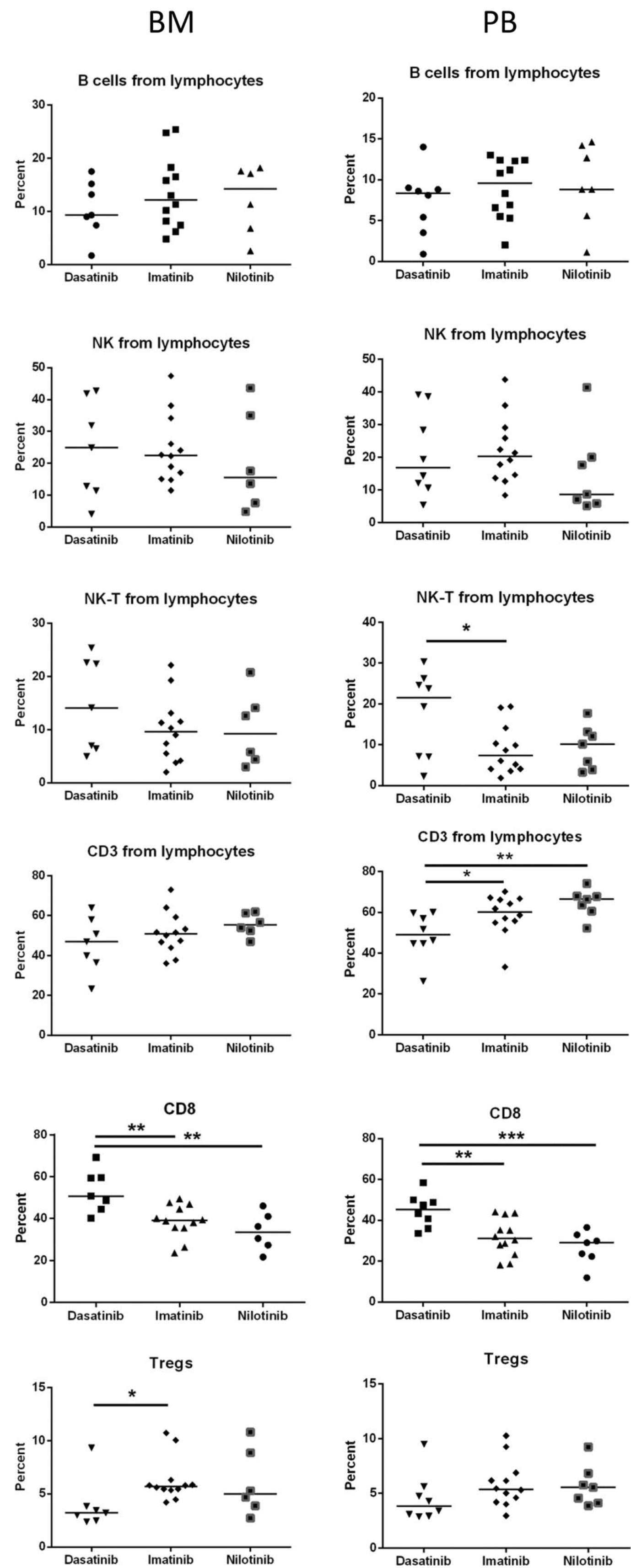
Discussion

Imatinib-induced BM lymphocytosis has previously been described in a small cohort of CML patients (Mustjoki et al. 2007). In this multi-centre study including 105 patients, we confirmed BM lymphocytosis phenomenon not to occur only with imatinib treatment, but also in patients treated first line with the second-generation TKIs dasatinib and nilotinib. The composition of the lymphocyte pool was similar in imatinib- and nilotinib-treated patients, but dasatinib patients had more activated CD8+ T cells in both PB and BM and less Tregs in BM samples.

During TKI therapy, the majority of CML patients achieve haematological remission within the first months of treatment. Therefore, it is expected that during the TKI therapy the percentage of lymphocytes exceeds the values at the diagnosis. However, we noticed that also when compared to healthy controls, CML patients had significantly increased amount of lymphocytes in the BM. The early increase in lymphocyte counts could also partly be related to myeloablation and eradication of leukaemia cells, but BM lymphocytosis seemed to persist during the TKI treatment when Ph+ cells were no longer detected in the BM in cytogenetic or molecular remission. Therefore, it is plausible that the TKI therapy induces the expansion of lymphocytes in the BM. Interestingly, in PB, lymphocytosis seems to occur only during dasatinib treatment, but not during imatinib or nilotinib treatment (Powers et al. 2011; Mustjoki et al. 2013a; Qiu et al. 2014). Therefore, the mechanisms inducing BM and PB lymphocytosis seem to be different, and dasatinib-associated PB lymphocytosis may relate to mobilization phenomenon, which has been shown not to occur during imatinib or nilotinib treatment (Mustjoki et al. 2013a).

Molecular response at 3-month time point has been reported and confirmed by many studies as an important prognostic factor (Beillard et al. 2003; Hanfstein et al. 2012; Neelakantan et al. 2013; Baccarani et al. 2013; Hanfstein et al. 2014). In our study, patients who achieved optimal early molecular response (*BCR–ABL1* < 10 %) had significantly higher lymphocyte counts when compared to patients who failed to achieve this milestone. Interestingly though, when later treatment responses were compared,

Fig. 5 Immunophenotype analysis of BM and PB samples. Immunophenotypic analysis of BM and PB samples was performed with multicolour flow cytometry at the 6-month time point after the therapy start. The proportion of CD19+ B cells, CD3-CD16/56+ NK cells, CD3+CD16/56+ NKT cells and CD3+ T cells was analysed from whole lymphocyte population. The proportion of CD8+ T cells was gated from whole CD3+ T cell population, and CD4+CD25+FoxP3+ Tregs were analysed from CD4+ T cells. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$



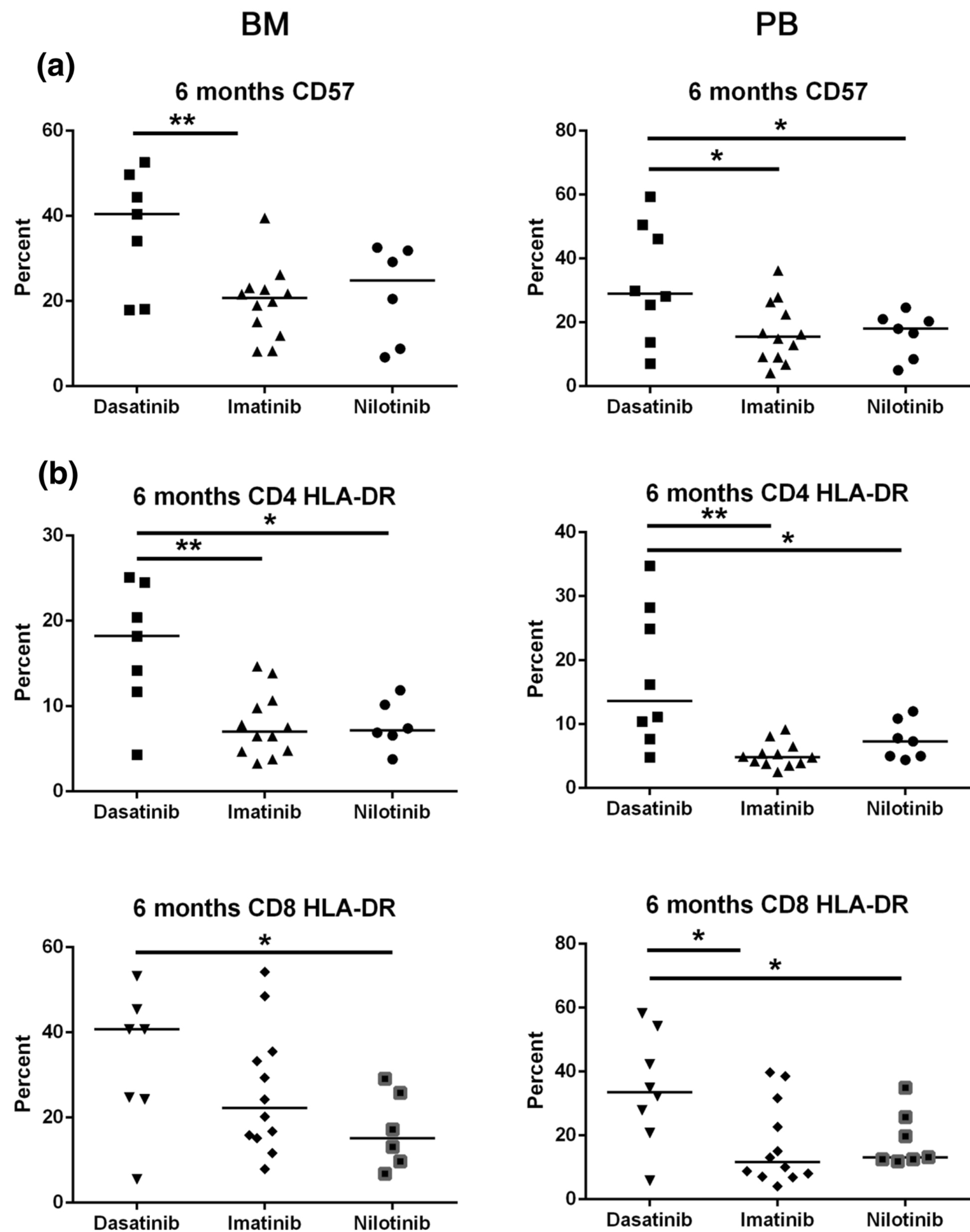


Fig. 6 Dasatinib-treated patients have increased amount of CD57+ and HLA-DR+ T cells in the BM and PB. The proportion of CD57+ T cells (**a**) and HLA-DR+ CD4+ and CD8+ T cells (**b**) was analysed with multicolour flow cytometry at 6-month time point. *T* test was used for the statistical analysis of the results. *$p < 0.05$; **$p < 0.01$

highest lymphocyte counts at 3-month time point were found in patients who failed to achieve major molecular remission (*BCR–ABL1* < 0.1 %) at 12-month time point [i.e. had a warning sign according to latest ELN recommendations (Baccarani et al. 2013)]. This can be partly explained by the state of immune reactivity and lack of immune homoeostasis in the sub-optimal group, but further studies are needed to clarify whether BM lymphocytosis is an independent prognostic factor.

Though BM lymphocytosis was observed during all different TKI therapies, there were significant differences in the composition of the expanded lymphocytic population. The biggest differences were observed in the number and phenotype of cytotoxic CD8+ T cells. When compared to imatinib- and nilotinib-treated patients, dasatinib-treated patients had significantly more CD8+ T cells in both the PB and BM. The HLA-DR expression, as a marker for activation, was also significantly increased in both the CD4+ and CD8+ T cells. This is in accordance with the previous publications showing LGL lymphocytosis occurring during dasatinib treatment (Mustjoki et al. 2009; Kreutzman et al. 2010; Powers et al. 2011). The decreased amount of Tregs has also earlier been reported to occur during dasatinib treatment (Rohon et al. 2010; Rohon 2012), but in the previous publications patients have mostly been treated second line with dasatinib thus having many prior therapies. Therefore, it is an important confirmation in this first-line treated patient cohort that the decrease in the number of Tregs

Table 3 CD62L expression in CD4+ and CD8+ T cells in CML patients treated with TKIs

	BM			PB		
	Imatinib	Dasatinib	Nilotinib	Imatinib	Dasatinib	Nilotinib
CD4CD62L						
Number of cases	12	7	6	12	8	7
Median (range)	82 % (60–93 %)	65 % (41–80 %)	83 % (71–92 %)	87 % (75–96 %)	75 % (49–85 %)	88 % (76–91 %)
	$p < 0.01$			$p < 0.01$		
		$p < 0.01$			$p < 0.05$	
CD8CD62L						
Number of cases	12	7	6	12	8	7
Median (range)	46 % (15–68 %)	23 % (9–37 %)	38 % (18–68 %)	62 % (16–84 %)	37 % (15–55 %)	50 % (37–79 %)
	$p < 0.01$			$p < 0.01$		

occurs already after 3–6 months of dasatinib treatment. As the overall lymphocyte balance in dasatinib-treated patients has shifted towards cytotoxic direction, it may have importance in the long-term control of the disease. For example, it has been reported that decreased CD62L expression, as observed in dasatinib-treated patients, could be considered as a marker for successful discontinuation of TKI treatment (Ohyashiki et al. 2012). However, it needs to be addressed in the future studies whether dasatinib-induced changes in the immune profile remain after the drug discontinuation.

To our knowledge, no detailed BM immunophenotyping analysis has been published during nilotinib treatment. Based on our small patient cohort, no significant differences in the basic lymphocyte subclasses including T, B, and NK cells as well as Tregs were observed between imatinib- and nilotinib-treated patients. In vitro studies have suggested that nilotinib may hamper the proliferation and suppressive capacity of Tregs, but this may occur at doses, which are not clinically relevant (Fei et al. 2010).

As a conclusion, BM lymphocytosis occurs during all current first-line TKI treatments, and it is associated with early molecular response. On the contrary, PB lymphocytosis occurs only during dasatinib treatment and is accompanied by the decreased amount of Tregs. In accordance with shared kinase inhibition spectrum, no marked changes in the immunoprofile are observed between imatinib- and nilotinib-treated patients.

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Compliance with ethical standards

Conflict of interest SM, KP, and HHjH have received honoraria and research funding from Novartis, Bristol-Myers Squibb, Pfizer and Ariad. OWB has received educational honoraria, grants, and research funding from Novartis, Bristol-Myers Squibb, Ariad, and Pfizer. HHjH has received honoraria from Novartis, Bristol-Myers Squibb, and Ariad. NCMLSG has received research funding from Bristol-Myers Squibb for the NordCML006 study.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments.

Informed consent Informed consent was obtained from all individual participants included in the study.

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