

Imatinib mesylate (Gleevec®) in advanced breast cancer-expressing C-Kit or PDGFR-β: clinical activity and biological correlations

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Background: Novel molecular therapies for metastatic breast cancer (MBC) are necessary to improve the dismal prognosis of this condition. Imatinib mesylate (Gleevec®) inhibits several protein tyrosine kinases, including platelet-derived growth factor receptor (PDGFR) and c-kit, which are preferentially expressed in tumor cells. We tested the activity of imatinib mesylate in MBC with overexpression of PDGFR or c-kit. Additionally, we sought to determine the biological correlates and immunomodulatory effects.

Patients and methods: Thirteen patients were treated with Imatinib administered orally at 400 mg p.o. b.i.d. (800 mg/day), until disease progression. All patients demonstrated PDGFR-β overexpression and none showed c-kit expression.

Results: No objective responses were observed among the 13 patients treated in an intention-to-treat analysis. All patients experienced disease progression, with a median time to progression of 1.2 months. Twelve patients have died, and the median overall survival was 7.7 months. No patient had a serious adverse event. Imatinib therapy had no effect on the plasma levels of the angiogenesis-related cytokines, vascular endothelial growth factor, PDGF, b-fibroblast growth factor, and E-selectin. Immune studies showed imatinib inhibits interferon-γ production by TCR-activated CD4⁺ T cells.

Conclusion: Imatinib as a single agent has no clinical activity in PDGFR-overexpressing MBC and has potential immunosuppressive effects.

Key words: c-kit expression, imatinib, immune-suppression, metastatic breast cancer

introduction

Metastatic breast cancer (MBC) is an incurable condition; current medical treatments have provided appropriate palliation but are unable to eradicate this disease [1, 2]. It is hoped that expanded knowledge of the biological bases of breast carcinogenesis and the mechanisms of cancer progression will lead to the development of more directed and effective treatment of this disease. The use of tyrosine kinase inhibitors (TKIs) may represent one such attempt at targeted 'translational' research.

Among these inhibitors, imatinib (STI571, Gleevec®) is the first successful, rationally developed, receptor-targeted agent

for chronic myelogenous leukemia (CML) [3]. Imatinib inhibits the constitutively active Bcr-Abl tyrosine kinase protein encoded by the fusion gene generated by the Philadelphia chromosome translocation, which is responsible for the pathogenesis of the disease [3]. Interestingly, recent *in vitro* studies have suggested a possible negative immunomodulatory effect of imatinib therapy that is likely related to the drug's effect on T-cell-specific kinases [4–6]. Imatinib also inhibits c-kit and platelet-derived growth factor receptor (PDGFR) kinases, with affinities similar to those described for the Bcr-Abl kinases [7, 8] C-kit encodes for KIT (CD117), a 145- to 160-kDa transmembrane receptor tyrosine kinase that plays an important role in the development of gastrointestinal stromal tumors, small-cell lung cancer, melanoma, and breast cancer [9–12].

PDGFR expression has been demonstrated in malignant breast tissue and surrounding stromal cells, including pericytes

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that support blood vessels [13, 14]. In preclinical studies, imatinib has shown antitumor activity in a breast carcinoma model, particularly in osteolytic bone metastases [15, 16]. Because breast cancer has been shown to variably express PDGFR and c-kit, we investigated the clinical activity of imatinib in women with MBC that expressed c-kit or PDGFR- β or both. Additionally, we sought to determine the biological correlates [17–19] and immunomodulatory effects associated with the administration of imatinib in women with breast cancer [4–6].

patients and methods

patient population

A prospective, open-label phase II study of imatinib for MBC was conducted at The University of Texas M. D. Anderson Cancer Center from September 2002 to February 2003. Eligible patients included those with measurable MBC, who were ≥ 18 years of age, had normal organ and marrow functions, had a score of ≤ 2 on the Eastern Cooperative Oncology Group performance status scale or a Karnofsky index of $>60\%$, had received at least one and not more than two prior chemotherapy regimens for metastatic disease, had received treatment with both an anthracycline and a taxane either as adjuvant or for advanced disease, and had a life expectancy of >12 weeks. Moreover, patients were required to have a prescreening assessment for c-kit (CD117) and PDGFR- β expression by the metastatic lesion as only patients with demonstrable expression of c-kit and/or PDGFR- β were considered for enrollment and treatment. Patients were excluded from the study if they had brain metastasis (or other symptomatic evidence of central nervous system disease) or if bone metastasis was the only disease site that could be evaluated. The Cancer Therapy Evaluation Program of the National Cancer Institute (CTEP/NCI) and M. D. Anderson's Institutional Review Board approved the protocol.

study design

Patients received imatinib mesylate [supplied by Novartis Pharmaceutical Corporation (Cambridge, MA) through CTEP/NCI] at a dose of 400 mg by mouth b.i.d. (800 mg/day) taken with a meal. Patients were treated continuously on a 4-week cycle. Treatment was discontinued for progression or severe toxicity. Dose reductions were permitted for patients with intolerable non-hematologic grade 2 toxicity or any grade 3 or 4 toxicity. If imatinib dose reduction was required, doses were reduced in 100-mg increments. Recurrent toxicity of similar severity resulted in another dose reduction, but patients who required more than two dose reductions or who had any delay of ≥ 2 weeks in scheduled therapy as a result of toxicity were withdrawn from the study. All patients were required to have absolute neutrophil counts $>1500/\mu\text{l}$ and platelet counts of $>75\ 000/\mu\text{l}$ in order to receive treatment on day 1 of each cycle of therapy.

Patients were reevaluated for response with standard imaging studies (computed tomography scans) every 8 weeks. In addition to a baseline scan, confirmatory scans were obtained 4 weeks following initial documentation of objective response. Definitions of response and disease progression were according to Response Evaluation Criteria in Solid Tumors (RECIST) [20].

pathology studies

Immunohistochemical staining was carried out on 4- μm sections cut from a representative paraffin block specimen of the invasive breast carcinoma. Immunostaining for c-kit and PDGFR- β was carried out in a DAKO autostainer with the LSAB-2 peroxidase kit (DAKO Corporation, Carpinteria, CA). Tissue sections were subjected to antigen retrieval using citrate buffer (pH 6.0) before immunostaining. Primary antibodies were

used against c-kit (DAKO cytoautomation, dilution of 1 : 400) and PDGFR- β (Santa Cruz biotechnology Inc., dilution of 1 : 50). For phosphorylated PDGFR- β , we carried out epitope retrieval using EDTA solution (pH 8.0) and stained the tissue sections with antibodies against phosphorylated PDGFR- β (Cell Signaling, Danvers, MA, 01923), dilution of 150, using the Biocare detection system. The chromogen utilized for detection of immunostaining was diaminobenzidine. Positivity for the markers was recognized as granular brown cytoplasmic staining. The extent of immunostaining was expressed as the percentage of positively stained tumor cells.

laboratory correlates

Peripheral blood samples from patients were collected at baseline, at 2–4 weeks after therapy and beyond 5 weeks after imatinib therapy. Whole blood was used for determining lymphocyte immunophenotypes by flow cytometry. Moreover, peripheral blood mononuclear cells (PBMCs) and plasma were separated from the whole blood for the detection of cytokine synthesis by activated T-cells and circulating biomarkers of angiogenesis, respectively.

measurement of biomarkers of angiogenesis

Plasma was analyzed in batches for interleukin (IL)-8, PDGF, tumor necrosis factor- α (TNF- α), transforming growth factor- β , E-selectin, vascular endothelial growth factor (VEGF), and basic fibroblast growth factor (b-FGF), using commercially available ELISA kits (R&D Systems, Minneapolis, MN) and the assays were run according to the manufacturer's instructions. Samples were run in duplicate, and the amount of each analyte was interpolated from a standard curve constructed with known concentrations of the particular analyte.

cell surface immunophenotypes of T cells

Total peripheral blood leukocyte counts and immunophenotypes of peripheral blood lymphocytes were determined pre- and postimatinib therapy. Percentages of total T cells (CD3⁺), helper T (CD4⁺), and suppressor/cytotoxic T (CD8⁺) cells were determined by flow cytometry. Absolute numbers of CD3⁺, CD4⁺, and CD8⁺ T cells were calculated to determine the effect of imatinib therapy.

intracellular cytokine synthesis by TCR-activated T-cells

The effect of imatinib therapy on T-cell function was studied in patients and the results compared with those of normal blood donors who were employees or graduate students at the M. D. Anderson Cancer Center. Normal donors of matched age were recruited by word of mouth, provided consent for a phlebotomy, and received a monetary compensation for their blood. PBMCs were activated with immobilized anti-CD3 antibody to measure cytokine syntheses by CD4⁺ T and CD8⁺ T cells, as we previously described [6].

statistical analysis

The primary objective of this study was to determine the efficacy of imatinib in MBC that demonstrated expression of CD117 (c-kit) and/or PDGFR as measured by objective tumor response. Secondary objectives included evaluating progression-free survival (PFS) of patients and the toxicity and tolerability of imatinib and defining plasma, tissue, and imaging surrogate end points of activity. In addition, we carried out correlative studies to identify potential surrogate markers of response.

The primary clinical end point was achievement of an objective tumor response (complete and partial responses) as determined by the RECIST [20]. To be of interest for further study, imatinib would have to produce a response rate of at least 30% and would have not been considered of interest for further study if the response rate was $<10\%$. Based on Simon's

optimal two-stage design [21] with false-positive and false-negative error rates of 10%, a maximum of 35 patients were to be accrued with one interim analysis planned after the first 12 patients were evaluated for response. The study would be continued to the second stage if at least two patients achieved an objective response. If the trial continued to the maximum of 35 patients, at least six responses would be required for imatinib to be considered of interest for further study.

PFS was measured from the start of treatment to the date of disease progression. Overall survival (OS) was measured from the start of treatment to the date of death from any cause or to the date of last follow-up. Estimates of time to progression and of OS were calculated using the Kaplan–Meier method with 95% confidence intervals (CIs).

Statistical differences in median levels of plasma cytokines, median percentages of leukocyte immunophenotypes, and median percentages of TCR-activated CD4⁺ T and CD8⁺ T cells that synthesized cytokines before and after initiation of therapy with imatinib were determined by the Mann–Whitney test. Evaluations were done at baseline, after 2–4 weeks and after 4 weeks. All specimens were compared with normal controls.

All analyses were carried out using SAS version 9.1 for Windows (SAS Institute, Cary, NC), S-Plus version 7 (Insightful Corp.)

results

patient characteristics

The characteristics of the 13 patients enrolled in this trial are presented in Table 1. All patients demonstrated PDGFR- β overexpression and none showed c-kit expression (Figure 1 A and B). Eight patients (61%) had visceral metastasis and seven patients (54%) had already received chemotherapy for metastatic disease.

response and survival

No objective responses were included in the intention-to treat analysis among the 13 patients treated. One of the patients

Table 1. Patients characteristics

Characteristics	n (%)
Sex	
Female	12
Male	1
Performance status (ECOG)	
0–1	12
2	1
Age, years	
Median (range)	52 (38–63)
Histology	
Ductal carcinoma	11
Lobular carcinoma	2
Site of metastasis	
Visceral	4
Bone/soft tissue	3
Both	4
Prior therapy for metastasis	
None	6
Chemotherapy	7
Hormonal therapy	4

ECOG, Eastern Cooperative Oncology Group.

discontinued treatment at home after a few doses and was initially considered not assessable. This allowed for an additional patient to be enrolled as for initial design. The subsequent information obtained on that patient suggested an early progression of disease and therefore 13 patients were included in the final intention-to-treat analysis. All patients experienced disease progression, with a median time to progression of 1.2 months (Figure 2). One patient with lobular carcinoma (hormone receptor positive) had biopsy-confirmed liver disease and was stable for >335 days. At 6 months, all but one patient had experienced a disease progression (PFS at 6 months = 8%, 95% CI = 1%, 51%). Twelve patients had died, and the median OS was 7.7 months (Figure 3). OS at 6 months was 62% (95% CI = 40%, 95%), and at 12 months it was 38% (95% CI = 19%, 77%).

toxicity

The toxicity profile of patients who received imatinib consisted mainly of general symptoms and gastrointestinal and hematologic toxicity (Table 2). Most adverse events were mild to moderate in intensity. The most severe events requiring dose reduction included vomiting and edema (Table 2).

correlative studies

serum cytokines. Imatinib therapy had no effect on the plasma levels of the angiogenesis-related cytokines, particularly

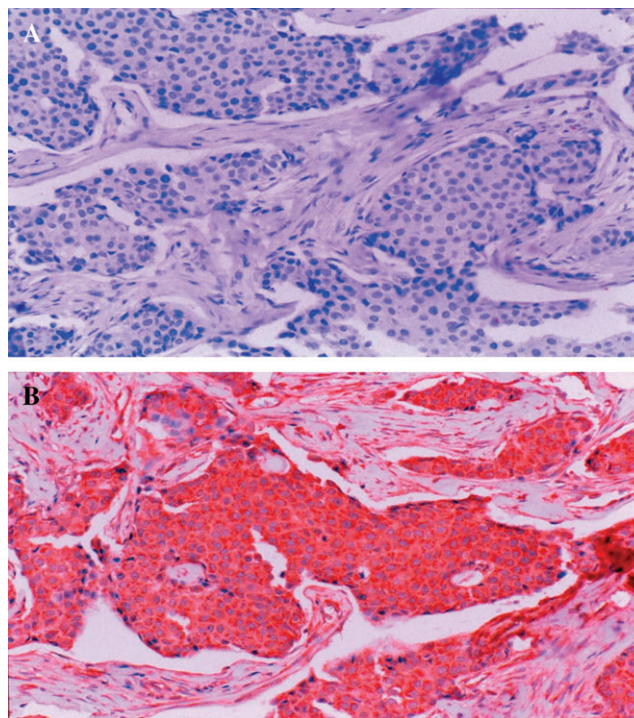


Figure 1. (A) A representative section of invasive ductal carcinoma immunostained for c-kit. Note that the tumor cells are entirely negative for c-kit. (B) A representative section of invasive ductal carcinoma immunostained for platelet-derived growth factor receptor (PDGF) β . The tumor cells show diffuse and strong cytoplasmic positivity for PDGF β .

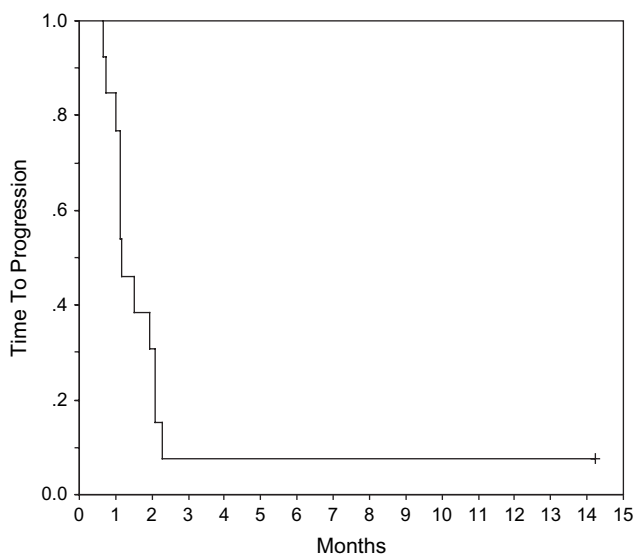


Figure 2. Kaplan–Meier representation of time to progression for patients with metastatic breast cancer treated with imatinib mesylate.

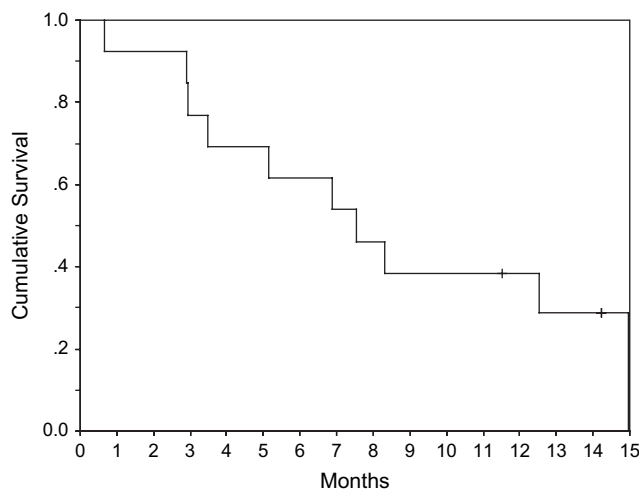


Figure 3. Kaplan–Meier representation of overall survival in the intention-to-treat analysis for patients with metastatic breast cancer treated with imatinib mesylate.

VEGF, PDGF, b-FGF, and E-selectin (Figure 4 A–D). There were no significant differences in the median levels of IL-8 and TNF- α before and after therapy with imatinib.

cell surface immunophenotypes of T cells. Imatinib had no effect on the percentage and absolute number of peripheral blood lymphocytes or on the percentages of CD3⁺ pan-T cells, CD4⁺ T, and CD8⁺ T cells. However, there was a noticeable, but not statistically significant, decline in the absolute number of total CD3⁺ pan-T cells by 2–4 weeks after therapy (1003/ μ l versus 839/ μ l), which reached a statistically significant decline after 4 weeks of therapy with imatinib (1003/ μ l versus 736/ μ l; $P = 0.035$) (Table 3).

T-cell activation by anti-CD3 through the TCR. The ability of CD4⁺ T and CD8⁺ T cells to synthesize IL-2, interferon

Table 2. Toxicity/adverse event with imatinib

	Any grade No. of patients (%)	Grade III/IV No. of patients (%)
Nausea	10	1
Diarrhea	7	1
Vomiting	6	4
Constipation	5	1
Edema	5	1
Stomatitis	3	0
Granulocytopenia	3	3
Infection without		
Myalgia	5	1
Rash/desquamation	3	0
Neutropenia	2	0
Thrombocytopenia	1	0
Anemia	1	0
Arthralgia	1	2
Dyspnea	1	1

(IFN- γ , TNF- α , or IL-10 in response to activation through the TCR was determined for 29 samples obtained from 13 patients. The breakdown of the samples consisted of 11 samples obtained at from patients at baseline, eight samples collected after 2–4 weeks of therapy, and 10 samples after >4 weeks of therapy with imatinib. After >4 weeks of therapy with imatinib, PBMC cultures had a decrease in the median percentage of TCR-activated CD4⁺ T cells, and not CD8⁺ T cells, that synthesized IFN- γ , which evolved into a statistically significant decrease in the median percentage of activated CD4⁺ T cells that synthesized IFN- γ (8.0% versus 3.6%; $P = 0.032$). Therapy with imatinib had no effect on the percentages of CD4⁺ T and CD8⁺ T cells that synthesized IL-2, TNF- α , or IL-10 following activation through the TCR with anti-CD3 antibody (Table 4).

discussion

The use of molecularly targeted therapies with mAbs or orally bioavailable, low-molecular weight TKIs has demonstrated the capacity of these therapies to improve the outcome of patients with both primary and MBC [22, 23]. The molecular ‘promiscuity’ of imatinib has expanded its therapeutic role in different tumor types supported through expression or persistent activation of c-kit and/or PDGFR tyrosine kinases [7, 14, 24–26]. Imatinib demonstrated extremely effective in gastrointestinal stromal tumors in which ~90% of tumor show c-kit abnormally express or mutated [27]. In invasive breast cancer, c-kit is rarely expressed while PDGFR is quite commonly overexpressed both in tumor and stromal cells, albeit at different levels [9, 10, 28–31]. These receptors and their downstream effectors trigger a cascade that regulates cell proliferation, differentiation, and survival [32]. In preclinical settings, the growth inhibitory effect of imatinib is related to a dose-dependent decrease of activation of PDGFR- β and Akt [33].

In this study, we demonstrated that imatinib as a single agent lacks clinical activity in patients with MBC selected for tissue

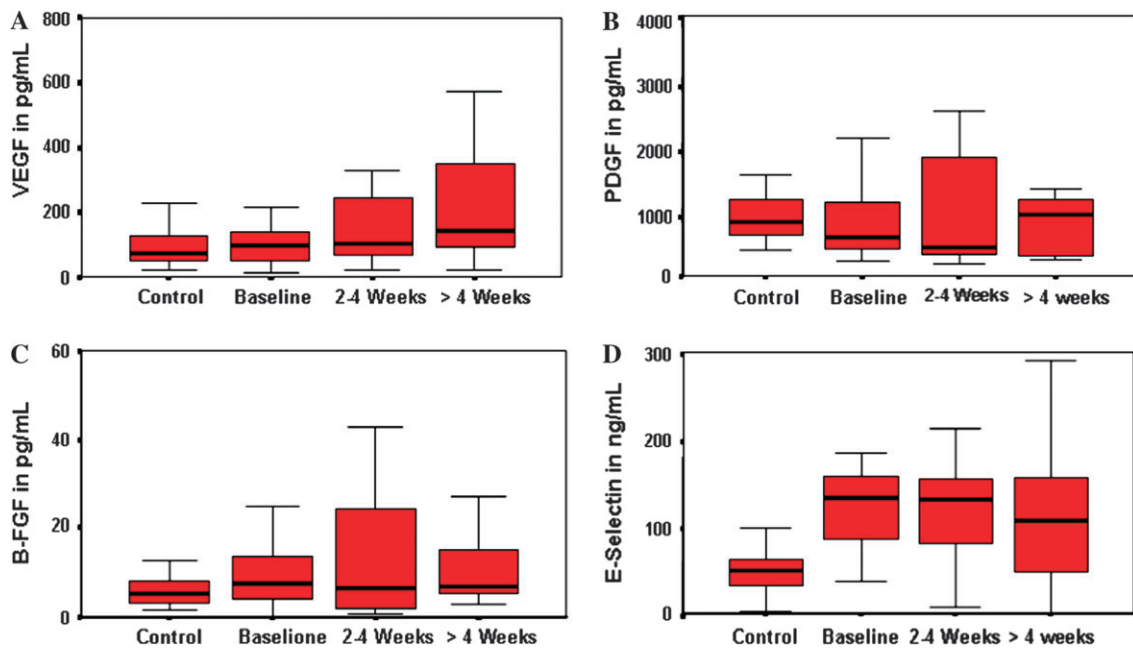


Figure 4. Plasma levels of (A) vascular endothelial growth factor, (B) platelet-derived growth factor, (C) basis fibroblast growth factor, and (D) E-selectin in metastatic breast cancer patients treated with imatinib mesylate.

Table 3. Effect of imatinib therapy on peripheral blood T-lymphocyte subsets

Parameter	Median (range) relative to initiation of imatinib therapy		
	Pretreatment	2–4 after	>4 weeks after
White blood cell per microliter	4.9 (3.1, 6.7)	4.2 (2.9, 6.3)	3.7 (1.7, 9.6)
% Lymphocytes	24.0 (6.0, 41.0)	22.0 (5.0, 69.0)	20.0 (7.0, 53.0)
% CD3 ⁺ T	69.9 (59.4, 81.2)	71.9 (47.5, 84.1)	70.1 (56.6, 86.2)
% CD4 ⁺ T	45.6 (20.0, 58.4)	46.5 (35.4, 57.7)	51.1 (29.1, 59.1)
% CD8 ⁺ T	22.2 (12.1, 39.7)	22.8 (12.0, 27.1)	20.9 (13.0, 32.5)
Lymph per microliter	1372 (868, 1876)	1176 (812, 1764)	1022 (476, 2688)
CD3 ⁺ T per microliter	1003 (580, 1460)	839 (591, 1233)	736^a (410, 1784)
CD4 ⁺ T per microliter	560 (252, 1064)	602 (348, 849)	5.2 (1.0, 18.3)
CD8 ⁺ T per microliter	298 (136, 744)	241 (198, 369)	235 (134, 482)

^aPretreatment *versus* >4 weeks after imatinib therapy, $P = 0.035$.

Table 4. Effect of imatinib therapy on cytokine syntheses by peripheral blood CD4⁺ and CD8⁺ T cells activated through the T-cell receptor

Cell type	Cytokine synthesized	Median percentages (range) of TCR-activated T-cell subsets synthesizing cytokines relative to start of therapy		
		Pretreatment	2–4 weeks after	>4 weeks after
CD8 ⁺ T	IL-2	4.4 (0.0, 11.7)	3.0 (0.3, 7.9)	3.0 (1.4, 13.0)
	IFN- γ	9.2 (1.1, 19.4)	6.9 (3.1, 14.8)	8.7 (2.3, 15.1)
	TNF- α	9.7 (1.4, 17.1)	6.6 (1.2, 18.0)	5.2 (1.0, 18.3)
	IL-10	2.5 (0.3, 6.2)	5.6 (1.5, 15.8)	6.9 (2.2, 10.2)
CD4 ⁺ T	IL-2	7.0 (0.1, 10.7)	3.7 (1.3, 8)	7.3 (1.7, 11.0)
	IFN- γ	8.0 (1.9, 16.2)	5.2 (3.2, 12.6)	3.6^a (0.56, 15.8)
	TNF- α	12.2 (0.4, 17.8)	9.0 (2.1, 19.8)	11.7 (3.2, 22.1)
	IL-10	3.3 (0.1, 5.0)	6.7 (1.6, 31.1)	4.2 (1.6, 23.4)

^aPretreatment *versus* >4 weeks after imatinib therapy, $P = 0.032$.

expression of either PDGFR- β (c-kit was not expressed). A few other clinical trials have tested imatinib either as a single agent or in combination regimens for MBC. Modi et al. [34] reported similar results with the same imatinib schedule as we used in 16 unselected patients; in their study, only one patient was c-kit positive and four were PDGFR positive. Toxicity in that study, mainly gastrointestinal, was significant in a heavily pretreated study group [34]. In our series, one patient with extensive liver involvement experienced a long stabilization (335 days) of disease. Unfortunately, a liver biopsy obtained at disease progression was impossible to repeat, making any speculation as to the reason for the stabilization useless.

In breast cancer, c-kit expression is found infrequently (1%–13%) but varies across the different tumor subtypes [28, 29]. The majority of c-kit-positive breast tumors appear to belong to the basal-like breast cancer subtype [35, 36]. By

mRNA expression, c-kit is one of the best basal-specific markers; however, by immunohistochemistry, many of the tumors that showed c-kit mRNA were not positive for c-kit protein expression [29]. C-kit-positive metastatic breast carcinoma accounts for <5% of breast carcinomas and appears to belong to the basal-like subtype of breast cancer. Mutational analysis of epidermal growth factor and c-kit receptor genes in this disease has been recently presented [37]. Expression of c-kit was detectable in ~25% of the tumors, but mutations predictive of responsiveness to imatinib were absent. PDGFRs are pivotal in peritumoral vasculature, stroma, and bone [13, 24, 25, 38, 39]. In another study, signaling through PDGF- β receptors was shown to increase interstitial fluid pressure and therefore to affect tumor cells' chemosensitivity [14]. Inhibition of this pathway by imatinib can enhance drug delivery to the tumor [40]. *In vitro* evidence from another study suggested that inhibition of the PDGF pathway would decrease the concentration of VEGF and other proangiogenic molecules [41]. The clinical significance of plasma levels of angiogenic factors like VEGF, b-FGF, PDGF, and E-selectin is presently unknown. In our study, we did not observe any statistically significant changes in the plasma levels of these angiogenesis-related molecules with imatinib therapy even though others have reported the ability of imatinib to decrease these factors.

Conflicting *in vitro* and *in vivo* results have been published on the negative immune-modulating effect of imatinib mesylate; this effect is likely related to a direct or indirect effect of the drug on T-cell-specific kinases [4–6, 42–44]. In preclinical tumor models, stimulation and proliferation assays showed these kinases to be inhibited, and a reduction in delayed hypersensitivity was demonstrated [5, 42]. On the other hand, whereas a positive effect of imatinib on bone marrow-derived antigen-presenting cells' restoring the responsiveness of tolerant T-cells from tumor-bearing hosts has been reported [44], we found imatinib to inhibit the synthesis of IFN- γ by TCR-activated CD4⁺ T cells. This was not unexpected as we previously found imatinib to interfere with the signaling of TCR-activated CD4⁺ T cells [6].

Retrospective analyses of patients with CML receiving imatinib demonstrated a relative low incidence of reactivation of herpes zoster infections [45]. In the current study, alteration in T-cell number and function as demonstrated by a decrease in the absolute number of CD3⁺ T cells and by TCR-activated CD4⁺ T cells synthesizing IFN- γ were observed. These data are consistent with other reports in hematological malignancies [4, 6] even though the consequences of immunological manifestations in patients with solid tumors are presently unknown. However, according to another study, no significant increase in adverse infectious events was reported at a 5-year follow-up in CML patients treated with imatinib [46].

In conclusion, our results indicate that targeting PDGFR with imatinib mesylate monotherapy in MBC is ineffective. Future studies should consider a careful selection of patients with c-kit overexpression (possibly basal-like breast cancers) and concomitant administration of chemotherapy because it appears unlikely to demonstrate single-agent activity. Nevertheless, our findings regarding immune functions should

be taken into account in cases of concomitant administration of imatinib with chemotherapy in previously treated patients with advanced disease [40].

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