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Research Article

Natural Killer Cell IFN-γ Levels Predict Long-term Survival with Imatinib Mesylate Therapy in Gastrointestinal Stromal Tumor–Bearing Patients

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Abstract

Clinical outcomes of gastrointestinal stromal tumor (GIST)–bearing patients treated with imatinib mesylate (IM) are variable. Other than the site of mutation within the *c-kit* gene, prognostic features of GIST remain undefined. IM can exhibit off-target effects such as triggering natural killer (NK) cell activity. We addressed whether NK cell functions could predict long term survival with IM. NK cell functions were followed up in 77 GIST patients enrolled onto two phase III trials. "Immunologic responders" were defined as patients whose NK cell IFN-γ values after 2 months of IM were higher than or equal to the baseline value at entry into the trial. The prognostic effect of IFN-γ on progression-free survival was assessed by a Wald test in a Cox regression analysis using the landmark method and stratified by trial and on the *c-kit* mutational status. Fifty-six patients were evaluable for the NK cell IFN-γ responses at baseline and 2 months. Their median follow-up for progression-free survival was 3.7 years. Thirty-four of 56 patients were immunologic responders to IM. In the Cox regression analysis, immunologic responders possessed a hazard ratio of progression or death equal to 0.29 (95% confidence interval, 0.12–0.70; P = 0.006) compared with nonresponders. Kaplan-Meier 2-year survival estimates were 85% for immunologic responders and 50% for nonresponders. Moreover, the immunologic response added prognostic value to the *c-kit* mutation. The NK cell IFN-γ production after 2 months of treatment could be considered an independent predictor of long term survival in advanced GISTs treated with IM. [Cancer Res 2009;69(8):3563–9]

Introduction

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors in the human digestive tract (1). Their molecular etiology has recently been unraveled and led to the paradigmatic use of specific therapies. Indeed, the sequencing of c-kit complementary DNA, which encodes a proto-oncogenic receptor tyrosine kinase (2) from GIST-bearing patients, revealed mutations in the region between the transmembrane and tyrosine kinase domains (2). All of the corresponding mutant KIT proteins were constitutively activated without the KIT ligand, stem cell factor. It was then postulated that GIST could originate from the interstitial cells of Cajal because the development of interstitial cells of Cajals is dependent on the stem cell factor-KIT interaction and because, such as GIST, these cells express both KIT and CD34. Somatic gain-of-function mutations of the c-kit proto-oncogene are found in 85% of GIST and mutations of the platelet-derived growth factor receptor (PDGFR)α chain were reported in 35% of the GISTs lacking the KIT mutations (3).

The 2-phenylaminopyrimidine compound STI571 [imatinib mesylate (IM); Gleevec; Novartis] has been initially designed to specifically block the ATP-binding site of BCR/ABL tyrosine kinase and found to inhibit the kinase activity of three kinases ABL, PDGFRα, and KIT (4–6). IM selectively inhibits c-kit tyrosine kinase activity and downstream activation of target proteins involved in cellular proliferation and survival. IM enables disease control including objective responses and stable diseases in >80% of GIST patients (7). Therefore, IM became the standard treatment of advanced GISTs. The questions as to the optimal dosing and duration of IM were then addressed in phase III trials. The European Organization for Research and Treatment of Cancer (EORTC) study (8) aimed at assessing dose dependency of response and progression-free survival (PFS) with IM concluded that a daily dose of 400 mg of IM should be recommended in the management of metastatic GIST patients. The French Sarcoma Group addressed in a prospective, randomized, multicentric phase III study (BFR14 trial) the duration of IM therapy and concluded that IM should be continued unless overt progression occurs (9).

Importantly, the mutational status of the KIT oncoproteins represents the most valuable predictive factor of clinical response to imatinib to date (3). Hence, patients whose tumors contained exon 11 KIT mutations had a longer survival than those whose tumors expressed either exon 9 KIT mutations or had no detectable kinase mutation. However, unexpected long-term responses to IM have been reported in GIST lacking the hallmark criteria of responses to such a targeted tyrosine kinase inhibitor (Le Cesne A. et al., abstract 10005 American Society of Clinical Oncology 2007).

In the search for additional prognostic factors, we addressed whether IM could exhibit functional side effects on KIT expressing the targets of the host. Indeed, tumor cell progression not only depends on cell autonomous tumor suppressive pathways but also on extrinsic immunologic barriers (10). Indeed, we highlighted an alternate mode of action of IM that is not tumor cell autonomous and involves—at least—host bone marrow–derived dendritic cells

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(DC). We unraveled the natural killer (NK) cell–dependent antitumor effects promoted by IM-treated DC in mouse tumor models resistant to the IM antiproliferative effects *in vitro* (11). These findings suggested the relevance of IM-mediated NK cell activation in patients bearing GIST devoid of KIT/PDGFRα mutations and displaying clinical responses to IM (11). It is noteworthy that IM-treated human DC were also endowed with NK cell stimulatory capacities *in vitro* (11). Because GIST tumor cells exhibit molecular features of NK cell sensitivity (11) such as loss of MHC class I molecules and expression of NKG2D ligands, we now address the role of the innate immune rsesponses in the response to IM and long-term survival in GIST patients followed up in the EORTC and BFR14 trials. Here, we show that IM does modulate NK cell responses in patients and propose an *in vitro* NK cell assay that could predict, at 2 months, the long-term response to IM independently of the exon 11 c-kit mutation. This finding may effect the current clinical management of GIST patients.

Materials and Methods

Patients and trials. The immunomonitoring studies of NK cell responses examined 34 GIST patients enrolled onto the EORTC phase III trial 62005 (8) and 43 GIST patients included in the French Sarcoma Group phase III clinical trial (BFR14; ref. 9), assessing dose and duration of IM, respectively. An informed written consent was obtained from patients according to the local ethical committee for the clinical and the immunologic studies. Heparinized blood was drawn from patients before treatment and after 2, 6, 12 mo of IM therapy. Characteristics of patients are depicted in Table 1. Tumor response was assessed by computed tomography scan, and the response was classified according to the Response Evaluation Criteria in Solid Tumors criteria every 3 mo after the beginning of treatment. Nonprogressive disease encompassed stable disease and partial or complete responses. About 18 healthy volunteers (sex- and age-matched with GIST patients) were used as controls for the immunologic parameters.

Table 1. Characteristics of patients

Gender (male/female)	44/33
Age	61 ± 13
Lymphocyte count (/mm^3)	1,647 ± 698
NK cells (% of lymphocytes)	11 ± 7
NK cell count (/mm^3)	175 ± 121
Primary tumor site	
Stomach	40%
Bowel	47%
Others	13%
Metastatic site(s)	
0	15%
1	60%
2	19%
3 or more	6%
c-kit mutation*	
Exon 11	51%
Exon 9	4%
None	17%
Not done	28%
Imatinib treatment	
400 mg	81%
800 mg	19%
Mean follow up (mo)	47 ± 18

*Assessed as reported in Emile, et al. (14).

Flow Cytometry Analyses

Peripheral blood was stained with the following antibodies: CD45-FITC (clone HI30), CD3-APC (clone UCHT) purchased from BD Pharmingen; NKp30-PE (clone Z25), NKp46-PE (clone BAB281), CD56-PC5 (clone N901), from Beckman-Coulter; and NKG2D PE (clone 149810) from R&D Systems. Fluorescence was acquired on a FACScalibur cytometer and subsequently analyzed with the CellQuest Pro software. In NKp30 cross-linking experiments, NK cells were incubated for 7 h with 2 μL of anti–CD107a-PE (clone H4A3; Pharmingen) in AIMV supplemented with 10 μmol/L monensin, and CD3/CD56 staining was performed after the cross-linking.

Assessment of *In vitro* NK Cell Effector Functions

Preparation of allogeneic monocyte-derived DC. Allogeneic monocyte-derived DCs were used for coculture with purified patients' NK cells. These DC were propagated from monocytes of normal volunteers harvested from leukapheresis as previously described (11). After 5 d, immature DC were frozen and one vial was subsequently thawed for each experiment.

Isolation and activation of peripheral NK cells. Peripheral blood mononuclear cells of GIST patients and NV were obtained by Ficoll-Hypaque (Amersham Pharmacia) density cushion centrifugation. For NKp30 cross-linking experiments, NK cells were purified (purity, >95%) with the human NK cells Easy Sep kit (Stem Cell Technologies) and seeded (10^5 NK per well) in 96-well Maxisorp plates (Nunc) coated with 2.5 μg/mL of mouse IgG2a anti-NKp30 (clone 210847; R&D Systems) or isotype control for 7 h. For the DC/NK cocultures, NK cells were purified with the Human NK cell Rosette Sep kit (Stem Cell Technologies) and incubated for 44 h with freshly thawed immature monocyte-derived DC. Maturation of DC was achieved by adding lipopolysaccharide (Sigma-Aldrich) in DC/NK cocultures at a final concentration of 5 μg/mL. Otherwise, NK cells were activated with 1,000 IU of rIL-2 (Chiron) for 44 h.

Cytokine quantification. After a 44-h DC/NK coculture or interleukin (IL)-2 stimulation, supernatants of NK cells were assessed for their IFN-γ levels using commercial ELISA kits (BD Biosciences/Pharmingen).

Cytotoxicity assays. *Purified* NK cells were used as effector cells in a standard 4 h chromium release assay against the $Na_2^{51}CrO_4$–labeled (Perkin-Elmer France) erythroleukemia K562. Experiments were conducted in triplicated wells at various effector/target ratios (10:1, 2:1, and 0.4:1).

Statistical Analyses

The Student's *t* test or Mann-Whitney test were used for comparison of the different groups of *in vitro* assays. For three or more groups, the one way ANOVA or Kruskall-Wallis tests were performed as appropriate, followed by Bonferroni or Dunn posttests, respectively. The prognostic effect of IFN-γ "immunologic responses" after 2 mo on PFS was assessed by a Wald test in a Cox regression analysis using the landmark method (12) and stratified by trial (i.e., BFR14 versus EORTC) and by *c-kit* mutational status. Thus, PFS was defined as the time from 2 mo after IM initiation to the date of progression or death, and censored at the date of last follow-up for those without events. The median follow-up was calculated using the inverted Kaplan-Meier method. A two-sided *P* value of 0.05 was considered significant.

Results

Impaired NKp30 functions in GIST patients. Flow cytometry analyses of peripheral blood NK cells revealed a profound down-regulation of the surface expression of the natural cytotoxicity receptor NKp30 (Fig. 1*A*) and to a minor extent of the alternate natural cytotoxicity receptor NKp46 (data not shown) in GIST patients at initiation of IM therapy compared with normal volunteers. Although found in other tumor models, the down-regulation of NKp30 expression was very severe (<25%) in 30% of GIST cases (Fig. 1*A*). The function of NKp30 was assessed by cross-linking the receptor for 7 h with anti-NKp30 monoclonal antibody

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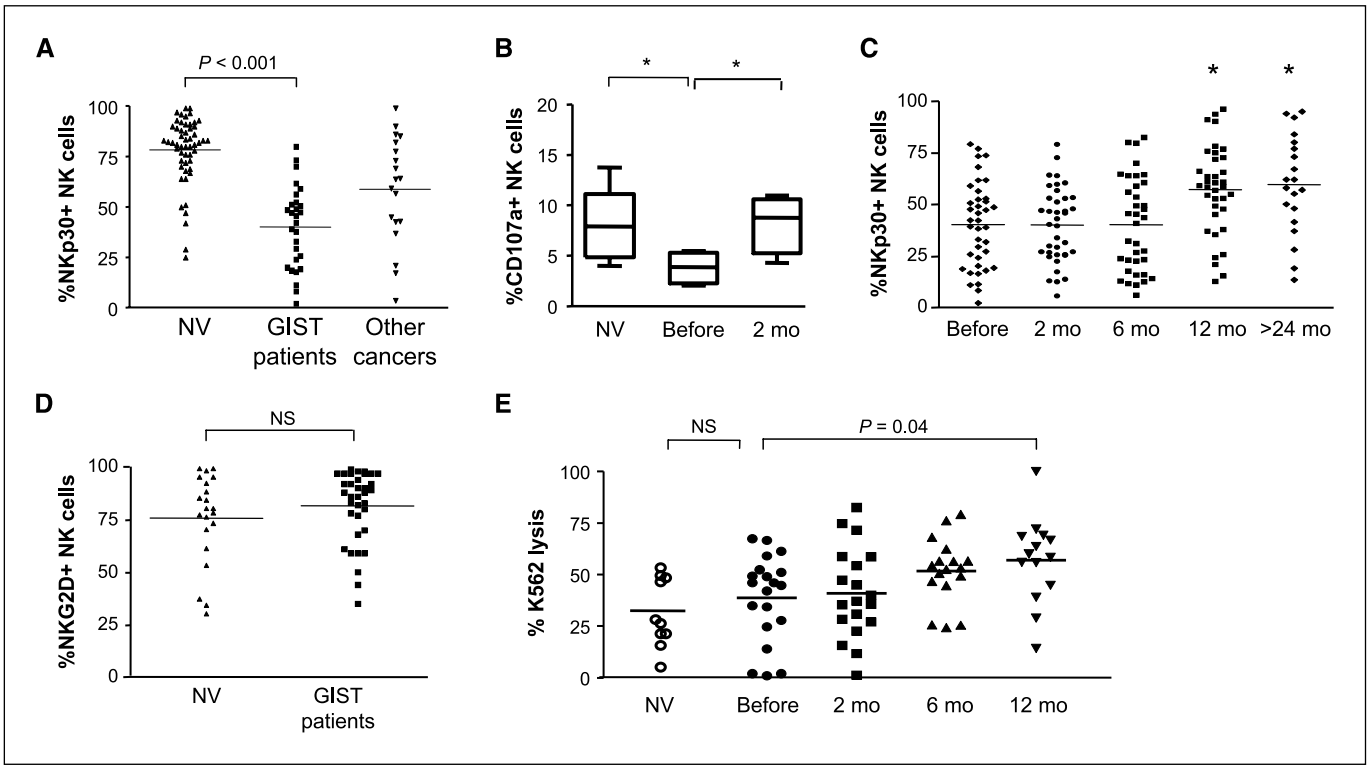

Figure 1. Modulations of NKp30 and NKG2D-related functions during IM therapy. NK cells from GIST (before and during therapy indicated in mo), or cancer patients or NV were analyzed by flow cytometry for their expression of NKp30 (*A* and *C*) or NKG2D (*D*). Each dot represents one individual. Cytotoxic activity of NK cells was assessed by CD107a-PE staining (*B*) or K562 lysis (*E*). *, $P < 0.05$. *NS,* not significant.

in vitro and by measuring the exocytosis of lytic granules using anti-CD107a monoclonal antibody. Membrane expression of CD107a was significantly reduced in GIST patients at diagnosis compared with NV (Fig. 1*B*). Although the expression levels of NKp30 significantly increased at late time points of IM therapy (Fig. 1*C*), the NKp30-dependent lytic potential was restored at early time points (Fig. 1*B*). Therefore, GIST patients exhibit profound but transient defects in NKp30 functions during IM therapy.

GIST patients display enhanced NKG2D functions. In contrast to NKp30, the lectin-like NK receptor NKG2D was normally expressed on circulating NK cells in GIST patients at diagnosis (Fig. 1*D*). Moreover, the NKG2D-dependent cytolysis of the erythroleukemia K562 was in reference ranges in GIST patients at presentation compared with NV (Fig. 1*E*). The dynamics of NKG2D-mediated functions was assessed at various time points during IM therapy for at least 1year. The spontaneous NK cell lysis against K652 augmented significantly by 1 year of IM therapy (Fig. 1*E*). Therefore, NK cells from GIST patients exhibit potent spontaneous and IM-induced NKG2D-dependent cytotoxicity.

Enhanced IFN-γ secretion after IL-2 and DC stimulation in GIST. Next, we assessed the capacity of NK cells from normal volunteers versus GIST patients to respond to two stimuli, IL-2 (Fig. 2) or maturing DC (mDC; Fig. 3). Production of IFN-γ by NK cells is mostly governed by IL-12 and to a minor extent by IL-2 (13). The main physiologic source of IL-12 is the mDCs. Moreover, we previously reported that the DC-mediated NK cell triggering for IFN-γ production was dependent on prestored granules of IL-12 delivered into the DC/NK cell synapse in murine and human cocultures *in vitro* (13). Therefore, we monitored the secretions of IFN-γ by NK cells after *in vitro* restimulation with the gold standard recombinant IL-2 (Fig. 2) or alternatively with mDC (to assess responsiveness to IL-12; Fig. 3). We used this *in vitro* coculture system (allogeneic DC together with GIST patients NK cells in the presence of lipopolysaccharide) to monitor the response of circulating NK cells to IL-12 secreted from mDC. Surprisingly, NK cells from GIST patients before IM treatment markedly responded to IL-2 (Fig. 2*A*) and mDC (Fig. 3*A*) compared with NV. Interestingly, IM rapidly augmented—by 2 months—the responsiveness to exogenous IL-2 (Fig. 2*A*) and mDC (Fig. 3*A*) *in vitro*.

Therefore, NK cells from GIST patients exhibited potent spontaneous and IM-induced IFN-γ secretion after *ex vivo* restimulation with IL-2 or mDC.

Clinical effect of IM-induced NK cell functions. The monitoring of NK cell functions during IM therapy revealed the potential relevance of three immunologic parameters.

The first criterion was the capacity of IM to boost the NKG2D-dependent lysis at 12 months. The time to progression was not significantly ameliorated in GIST exhibiting IM-induced enhanced killing functions versus the other GIST (data not shown).

The second criterion was the capacity of IM to boost the IL-2–dependent IFN-γ production at 2 months, which could be assessed for 38 patients. Two cohorts of GIST patients could be readily distinguished, i.e., those for whom the IL-2–induced IFN-γ production was equal or higher at 2 months compared with baseline (Fig. 2*B, right*) versus those for whom NK cells failed to respond to IL-2 (Fig. 2*B, left*). We studied the time to progression of these 38 patients that benefited from a median follow-up of 40 months in both cohorts of GIST (Fig. 2*B*). The time to progression was not statistically longer in patients with enhanced IL-2–induced IFN-γ production (Fig. 2*C*; log-rank test, $P = 0.19$).

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The third criterion was the capacity of IM to boost the mDC-dependent IFN-γ production. Fifty-six patients were evaluable for the NK IFN-γ responses at 0 versus 2 months (43 patients in BFR14, 13 patients from EORTC trial). Their median follow-up for PFS was 3.7 years, and 36 of 56 patients had progressed during follow-up. "Immunologic responders" were defined as patients whose NK cell IFN-γ values were higher or equal to the baseline value at entry in the trial. Thirty-four of 56 were found to be immunologic responders (Fig. 3*B, right*). The 11 "nonimmunologic responders" were defined as patients whose IFN-γ values had decreased after 2 months compared with the baseline value (Fig. 3*B, left*). The prognostic effect of immunologic responses (based on NK cell IFN-γ production after mDC stimulation) at 2 months of IM on PFS was assessed by a Wald test in a Cox regression analysis using the landmark method and stratified by trial (i.e., BFR14 versus EORTC). In the Cox regression analysis stratified by trial, the immunologic responders possessed a hazard ratio of progression or death equal to 0.29 (95% confidence interval, 0.12–0.70; $P = 0.006$) compared with non immunologic responders. Kaplan-Meier 2-year survival estimates were 85% (95% confidence interval, 73–97%) for immunologic responders and 50% (95% confidence interval, 29–71%) for non immunologic responders (Fig. 3*C*).

From these 56 patients, 38 patients have been evaluated for *c-kit* mutation status (14) at exon 11, which predicted the response to IM (3). Twenty nine patients exhibited a tumor presenting with an exon 11 mutation, whereas 9 harbored a tumor lacking an exon 11 mutation ("nonexon 11"). In a Cox regression analysis stratified by trial, the nonexon 11 tumor-bearing patients had significantly poorer PFS compared with the exon 11 *c-kit*–mutated tumor-bearing patients [hazard ratio of progression or death equal to 0.32 (95% confidence interval, 0.11–0.98; $P = 0.046$)]. Two-year survival estimates were 56% (95% confidence interval, 23–88%) for nonexon 11 tumor–bearing patients and 86% for exon 11 *c-kit*–mutated tumor–bearing patients (95% confidence interaval, 73–99%).

To assess whether NK cell IFNγ production after stimulation with mDC at the host level added a prognostic value to the *ckit* mutation status of the tumor, we analyzed *ckit* mutation status as a stratification factor in the Cox regression model together with the trial. Immunologic responders still presented a hazard ratio of progression or death equal to 0.30 (95% confidence interval, 0.28–0.87; $P = 0.03$) compared with nonimmunologic responders.

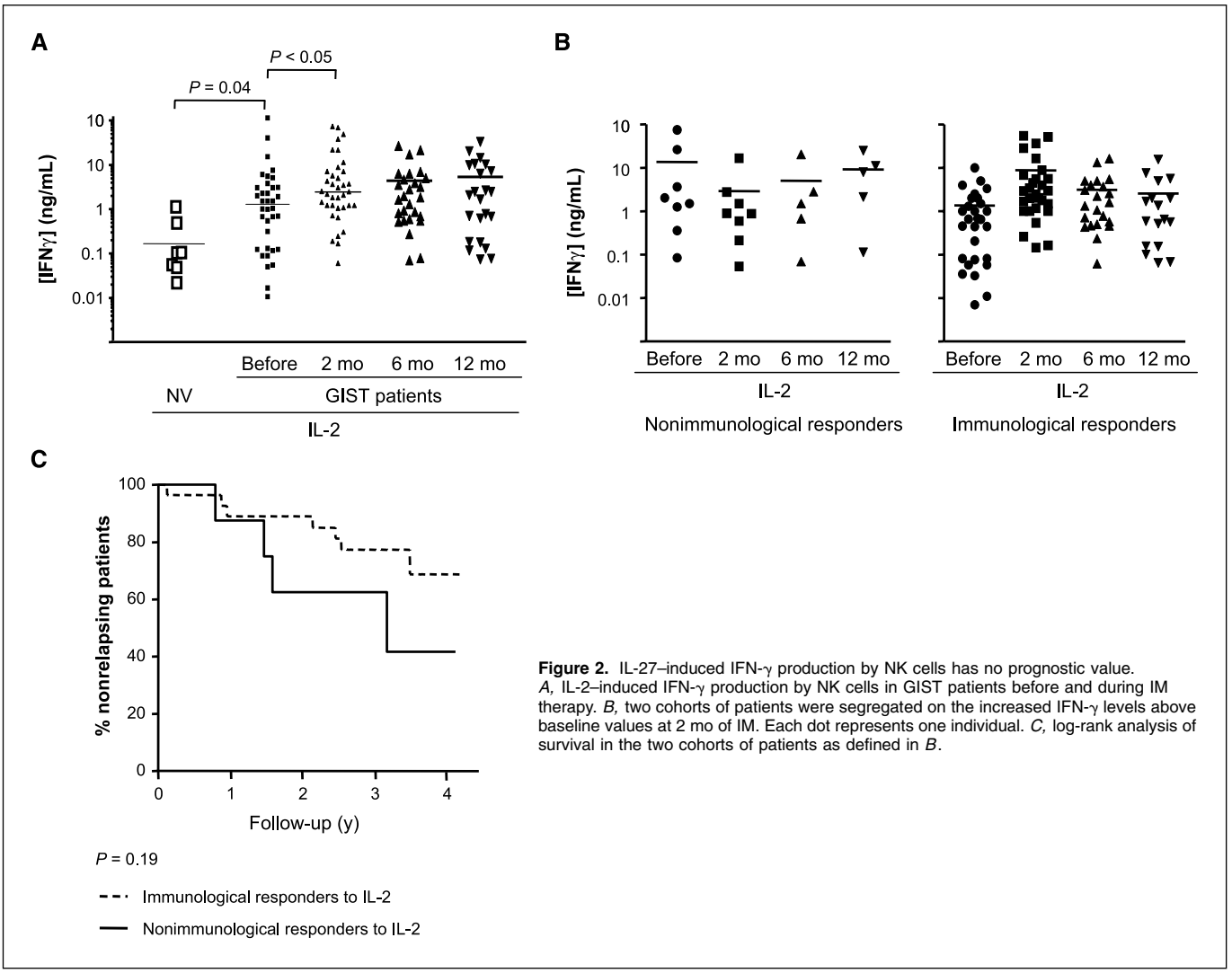

Figure 2. IL-27–induced IFN-γ production by NK cells has no prognostic value. *A,* IL-2–induced IFN-γ production by NK cells in GIST patients before and during IM therapy. *B,* two cohorts of patients were segregated on the increased IFN-γ levels above baseline values at 2 mo of IM. Each dot represents one individual. *C,* log-rank analysis of survival in the two cohorts of patients as defined in *B*.

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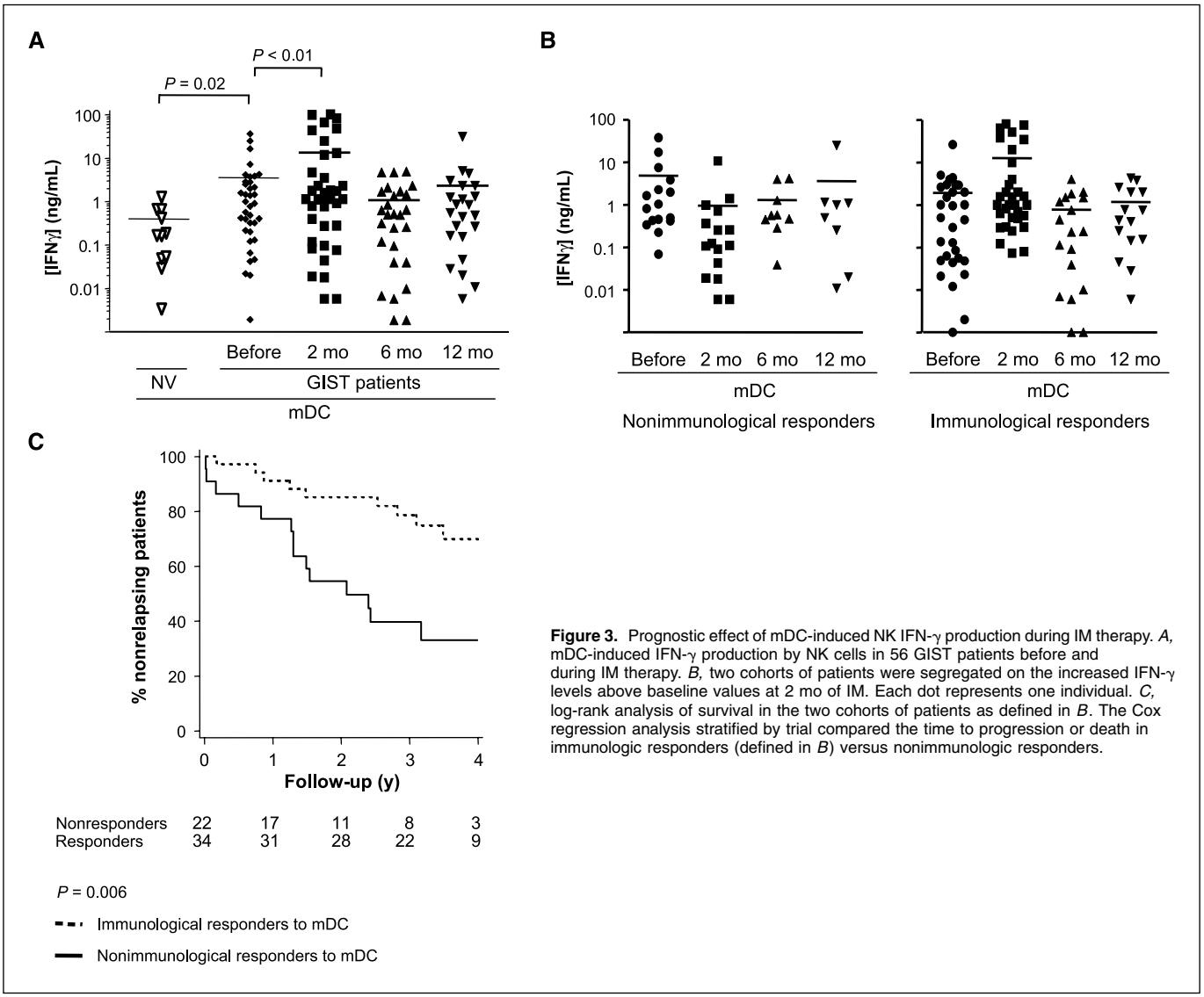

Figure 3. Prognostic effect of mDC-induced NK IFN-γ production during IM therapy. *A,* mDC-induced IFN-γ production by NK cells in 56 GIST patients before and during IM therapy. *B,* two cohorts of patients were segregated on the increased IFN-γ levels above baseline values at 2 mo of IM. Each dot represents one individual. *C,* log-rank analysis of survival in the two cohorts of patients as defined in *B*. The Cox regression analysis stratified by trial compared the time to progression or death in immunologic responders (defined in *B*) versus nonimmunologic responders.

We confirmed this original finding by adding 21 additional patients for whom the value of NK cell IFN-γ production after stimulation with mDC was only available at 2 months (but was missing at baseline before IM therapy). To classify these latter cohort into immunologic versus nonimmunologic responders, we set up a cutoff value at 0.7 ng/mL because 95% of normal volunteers produced <0.7 ng/mL of IFN-γ (Fig. 3)?. Therefore, 77 GIST (the first 56 and the additional 21 cases) could be segregated into immunologic versus nonimmunologic responders (Table 2). Moreover, 4 prognostic factors predicting late resistance have been

Table 2. Prognostic factors for PFS

Parameter	Median PFS (mo), logrank *P*	Multivariate analysis	
		Hazard ratio	*P*
KIT mutation: exon 11 vs others	60 vs 33, *P* = 0.19	—	—
Granulocyte count: normal vs high	61 vs 31, *P* = 0.07	—	—
Largest lesion at entry: <12 vs >12 cm	49 vs 44; *P* = 0.83	—	—
Primary tumor: gastric vs other location	Not reached vs 44, *P* = 0.68	—	—
Dose of imatinib: 800 vs 400 mg	Not reached vs 36, *P* = 0.13	—	—
NK IFNγ 2 mo vs baseline: increased vs decreased	Not reached vs 31, *P* = 0.002	0.339	0.003

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reported (15), namely large tumor size (>12 cm), high baseline blood granulocyte count at entry (>5.10^9/L), low dosing of IM (400 mg daily), and extragastric location of the primary tumor. In a multivariate analysis using Cox model, the immunologic response to mDC was the only independent prognostic factor for PFS (hazard ratio, 0.339; $P = 0.003$), whereas *c-kit* mutation status, diameter of the largest lesion, granulocyte count, IM dosing, and primary tumor site were not retained by the model (Table 2). These results indicate that the innate immune response is a major and independent predictor for PFS in patients with advanced GIST receiving IM.

Discussion

We aimed at investigating the predictive role of host-derived parameters namely NK cell effector functions in the long-term survival mediated by IM therapy in 77 GISTs. The 1-year immunomonitoring follow up of NK cell effector functions in 2 phase III trials revealed the enhancement of the NKG2D-dependent cytotoxicity and IL-2/IL-12–dependent IFN-γ production. However, enhanced NKG2D-dependent lysis observed at 1 year of IM therapy did not effect on survival (data not shown). Although the late immunologic events might just be a consequence of tumor regression, the early modulations of innate immune responses are of utmost importance in that they could allow to select patients benefiting from continuous IM therapy or, conversely, to redirect patients toward alternative therapeutic options. Interestingly, we found that the immunologic response defined as the IM-induced IFN-γ secretion after *ex vivo* stimulation by mDC is a valuable predictive and independent indicator of long-term survival (Fig. 3*C*).

Cancer is widely considered to be a cell-autonomous genetic disease that results from alterations in oncogenes, tumor-suppressor, and genome stability genes. However, cancer is also a disease in which the tumor cell microenvironment, the stroma, and immunity play a major role. Indeed, for the development of full-blown neoplasia, cancer cells have to overcome intrinsic (cell-autonomous) as well as extrinsic (immune-mediated) barriers to oncogenesis (10). NK cells have been recently involved in the host-mediated control of cancer (16–19). The renaissance of NK cells in tumor immunosurveillance can be attributed to the discovery of stress-induced ligands for NK cell–activating receptors (20) and the relevance of MHC class I/Killer Inhibitory Receptor interactions in mismatched hematopoietic transplants causing NK cell–mediated graft versus leukemia effects (16). In acute myeloid leukemia (AML), impaired NKp30-mediated NK cell lysis has been associated with early relapse (21). However, in contrast to AML, GIST tumor cells did not seem to be recognized in a NKp30-dependent manner but rather through NKG2D receptor (11).[9] Surprisingly, our data reveal that NKG2D-dependent functions are not critical for the 5-year event-free survival (data not shown), suggesting that the role of NK cells might not be at the effector phase of the immune responses but rather at the regulatory phase, in the lymph nodes.

Lymph nodes contain several subsets of DCs that are critical links between innate and cognate immune responses (22). The DC/NK cell cross-talk has been recently involved in the host-mediated control of cancer (17, 19). The capacity of NK cells to control DC maturation or killing is a cornerstone regulatory event for cognate immune responses (23, 24). The NK-mediated DC activation is mediated through IFN-γ and tumor necrosis factor (TNF)-α *in vitro* (25). How IM is operating remains speculative. Our unpublished data do not support the fact that IM acts on GIST cells (a GIST cell line exhibiting an exon 13 c-kit mutation) to sensitize NK cell attack. We have reported that IM acts on the c-kit signaling pathway of mouse myeloid DC subsets to promote NK cell IFN-γ secretion and cytotoxicity (11). This DC-mediated NK cell triggering might occur in lymph nodes (rather than tumor; beds refs. 26, 27) and lead to the exit of NK cells from lymph nodes and recirculation in the blood (compartment where we performed our immunomonitoring studies). Supporting this hypothesis, our results indicate that mDC-dependent IFN-γ production by NK cells is higher after IM therapy and dictates the clinical outcome under IM. mDC can indeed form active immunologic synapses with resting or preactivated NK cells through a mechanism involving cytokines and cell to cell contacts (13, 28). It is conceivable that in addition to IM, other pathophysiologic conditions might deliver TLR4 ligands (such as HMGB1; ref. 29) in tumor-draining lymph nodes that could recapitulate such a productive DC/NK cell cross-talk. Moreover, our unpublished data favor the hypothesis whereby IFN-γ and TNF-α, resulting from the IM-induced DC/NK cell cross talk, act in concert to block the proliferation of GIST cells. It is also conceivable that IFN-γ and IM might operate together against GIST neoangiogenesis, as suggested by previous reports (5, 30).

For historical reasons, drug discovery programs for cancer therapy have neglected the possibility that immune reactivity might contribute to the efficacy of treatment (31, 32). Although cancer chemotherapy and radiotherapy is often viewed as a strategy that mainly affects tumor cells, accumulating evidence indicates that, depending on the lethal stimulus, tumor cell death can elicit a specific immune response that contributes to the therapeutic effect (29, 33, 34). Other drugs may have side effects that stimulate the immune system, by transient lymphodepletion (7), by the subversion of immunosuppressive mechanisms (33, 35), or by direct or indirect stimulatory effects on immune effectors (36, 37). Moreover, vaccination against cancer-specific antigens can sensitize the tumor against subsequent chemotherapeutic treatment (38–43). Our data bring up the first evidence of (*a*) a host-derived factor in dictating GIST outcome during IM therapy (3, 44, 45), of (*b*) a prognostic effect of NK cell functions in solid tumors, and of (*c*) an immunologic "off-target" effect on NK cells of a tyrosine kinase inhibitor. A larger patient series will be needed to validate the prognostic value of an augmented NK cell IFN-γ production at 2 months of IM therapy and to establish the added value of this immunologic parameter over that of the *c-kit* mutation status or ultrasonography (46).

[9] Our unpublished data.

Disclosure of Potential Conflicts of Interest

J.Y. Blay: Commercial research grant, Novartis Pharmaceuticals. A. Le Cesne: Consultant, Novartis Pharmaceuticals. L. Zitvogel: Commercial research grant, Novartis Pharmaceuticals. The other authors disclosed no potential conflicts of interest.

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