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Sustained Molecular Response With Interferon Alfa Maintenance After Induction Therapy With Imatinib Plus Interferon Alfa in Patients With Chronic Myeloid Leukemia

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ABSTRACT

Purpose
Imatinib induces sustained remissions in patients with chronic myelogenous leukemia (CML), but fails to eradicate CML stem cells. This is of major concern regarding the issues of cure, long-term imatinib tolerability, and imatinib resistance. We therefore asked whether interferon alfa-2a (IFN) alone could maintain molecular remissions achieved by a prior combination therapy with imatinib and IFN.

Patients and Methods
Imatinib therapy was stopped in 20 patients who had concomitantly been pretreated with imatinib and IFN for a median of 2.4 years (range, 0.2 to 4.8 years) and 2.5 years (range, 0.2 to 4.9 years), respectively. After imatinib discontinuation, remission status was monitored monthly by quantitative analysis of the peripheral-blood *BCR-ABL* mRNA levels using real-time polymerase chain reaction. Proteinase-3 expression and proteinase-3–specific cytotoxic T cells (CTLs) were longitudinally measured to assess putative markers of IFN response.

Results
With a median time of 2.4 years after imatinib withdrawal (range, 0.5 to 4.0 years), 15 (75%) of 20 patients remained in remission. The number of patients in complete molecular remission increased under IFN from two patients at baseline to five patients after 2 years. Relapses occurred in five patients within 0.4 years (range, 0.2 to 0.8 years), but patients underwent rescue treatment with imatinib, re-establishing molecular remission. IFN therapy was associated with an increase in the expression of leukemia-associated antigen proteinase 3 and induction of proteinase-3–specific CTLs.

Conclusion
Treatment with IFN enables discontinuation of imatinib in most patients after prior imatinib/IFN combination therapy and may result in improved molecular response. Induction of a proteinase-3–specific CTL response by IFN may contribute to this effect.

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INTRODUCTION

Imatinib mesylate (Imatinib; Novartis Pharma, Basel, Switzerland) selectively inhibits the BCR-ABL tyrosine kinase as the causative genetic aberration in chronic myeloid leukemia (CML).[1,2] Imatinib induces sustained clinical responses in the vast majority of patients with chronic-phase CML.[3] However, primitive $CD34^+$ CML precursor cells may be insensitive to imatinib[4] and to the more potent second-generation ABL tyrosine kinase inhibitors (TKI) nilotinib[5] and dasatinib,[6] leading to persistence of *BCR-ABL*–positive cells. Detection of residual BCR-ABL mRNA transcripts in the majority of patients with CML despite long-term imatinib treatment supports the notion of disease persistence after TKI therapy.[3,7] Frequent relapses after imatinib discontinuation even after complete molecular remission (CMR) have been reported.[8] Hence, to prevent relapse and disease progression, indefinite imatinib therapy is currently the recommended standard.[9] However, permanent TKI intake also raises concerns regarding the evolution of drug resistance,[10] long-term safety and tolerability,[11] compliance issues,[12] and costs.[13] Significant efforts are being undertaken to overcome this dilemma. For example, specific means of targeting CML stem cells may offer the chance to overcome disease persistence, discontinue imatinib, and achieve cure. To date, allogeneic stem-cell transplantation is considered

the only curative therapy of CML, because this procedure eliminates CML stem cells via the graft versus leukemia effect. [14]

In an important minority of patients with CML, however, interferon alfa (IFN) may also generate long-term remissions,[15,16] permitting discontinuation of the drug[17] by targeting CML precursors immunologically. IFN stimulates autologous cytotoxic T cells (CTLs) to specifically recognize BCR-ABL– or BCR-ABL–dependent antigens.[18] One of the BCR-ABL–dependent antigens is the serine protease, proteinase-3 (myeloblastin). PR1-specific CTLs recognize a nonapeptide of proteinase-3 in an HLA-A0201–restricted manner and are capable of specifically eliminating CML progenitors.[19] The presence of PR1-CTL was found to be specifically associated with responsiveness to IFN in CML.[20-22] Elicitation of immunity to CML-specific self-antigens such as proteinase-3 would explain why IFN treatment can be terminated in complete cytogenetic responders without losing cytogenetic remission.[23]

We have previously shown that IFN, but not imatinib, elicits PR1-specific CTL responses in CML.[21] On the basis of this finding, we suggested that a parallel or consecutive combination therapy of imatinib and IFN might induce cytotoxic and immunologic modes of remission. We sought to evaluate the long-term outcome of patients with CML on IFN maintenance therapy after imatinib/IFN combination therapy, considering the depth of molecular remission.

PATIENTS AND METHODS

Patients and Treatment

Twenty patients with chronic-phase CML (14 men and six women; median age at diagnosis, 40 years; range, 24 to 66 years) have been investigated after written informed consent was obtained. Thirteen patients were at low, six were at intermediate, and one was at high risk according to the Euro score. First-line therapy consisted of a combination of imatinib (400 mg orally daily) and concomitant subcutaneous injections of either recombinant IFN (n = 3; 3 × 3 million U weekly) or 90 to 135 μg of pegylated IFN-α-2a (n = 17; Pegasys; Roche, Basel, Switzerland) given once every 1 to 3 weeks according to efficacy and tolerability. IFN/imatinib combination treatment was administered within two multicenter trials, a phase II Pegasys/imatinib combination therapy trial and the German CML study IV (NCT00055874). The time point of imatinib discontinuation is referred to as baseline. Reasons for discontinuation of imatinib were request of the patient and/or imatinib intolerance. Nineteen patients experienced long-lasting grade 1 to 2 or acute grade 3 side

Table 1. Individual Patient and Response Details

			Imatinib/IFN Induction					IFN Maintenance					
						Depth of Remission at Baseline						Best Molecular Response After Baseline (BCR-ABL IS, %)	
Patient	Sex and Age at Diagnosis (years)	Euro Risk Score	IM (years)	IFN (years)	Type of IFN	Cytogenetic	Molecular (BCR-ABL IS, %)	Years Off IM	Most Recent IFN Dose	Time to Relapse (years)*	Adverse Effects (grade)	During First Year	During Second Year
1	F, 24	Intermediate	2.4	2.5	PEG	CCR	0	2.8	135 μg/14 d	—	None	0	0
2	M, 35	Low	2.3	2.4	PEG	CCR	0	2.1	90 μg/21 d	—	Cutaneous sarcoidosis (2), fatigue (1)	0	0
3	M, 53	Low	1.5	1.6	PEG	CCR	0.0053	2.9	135 μg/21 d	—	None	0	0
4	M, 52	Intermediate	3.1	2.9	PEG	CCR	0.0044	1.4	135 μg/21 d	—	None	0.0053	0.0076
5	M, 28	Low	4.5	4.6	PEG	CCR	0.0071	2.5	135 μg/10 d	—	Fatigue (1)	0	0
6	M, 60	Intermediate	2.4	2.1	Rec	CCR	0.0075	3.6	3 × 3 million U/wk	—	None	0.0041	0
7	M, 49	Low	4	3.9	PEG	CCR	0.023	1.5	180 μg/14 d	—	None	0.015	0.025
8	M, 52	Intermediate	2.6	2.7	PEG	CCR	0.037	2.0	135 μg/21 d	—	Fatigue (1)	0.0045	0.0041
9	F, 54	Low	2.9	3.0	PEG	CCR	0.012	2.0	135 μg/14 d	—	None	0	0
10	M, 65	Low	1.0	1.2	PEG	CCR	0.048	3.7	135 μg/21 d	—	Fatigue (1)	0.0088	0.0044
11	F, 23	Low	2.2	2.3	PEG	CCR	0.026	2.3	135 μg/10 d	—	None	0.0070	0.0076
12	F, 31	Intermediate	1.8	1.7	PEG	CCR	0.031	2.4	135 μg/14 d	—	Diarrhea (1)	0.035	0.022
13	M, 51	High	3.3	3.2	PEG	CCR	0.016	3.9	Stop after 44 months	—	None	0	0
14	F, 40	Low	2.7	3.6	Rec	CCR	0.053	2.6	Stop after 31 months	—	Fatigue (1)	0	0
15	M, 32	Low	0.2	0.1	PEG		35	3.1	180 μg/7 d	—	Fatigue (1)	0.097	0.039
16	M, 32	Low	2.1	1.8	Rec	CCR	0.016	0.7	3 × 3 million U/wk	0.4	None	NA	NA
17	M, 62	Low	1.9	2.0	PEG	CCR	0.050	1.2	135 μg/7 d	0.8	Flu-like symptoms (1)	NA	NA
18	M, 35	Low	4.9	4.8	PEG	CCR	0.011	0.5	135 μg/14 d	0.4	None	NA	NA
19	F, 28	Low	3.0	3.0	PEG/Rec†		0.31	1.0	5 × 3 million U/wk	0.6	None	NA	NA
20	M, 74	Intermediate	0.8	0.7	PEG	CCR	0.21	3.5	135 μg/7 d	0.2	None	NA	NA

Abbreviations: IFN, interferon alfa 2a; IM, imatinib; IS, international scale; F, female; PEG, pegylated; CCR, complete cytogenetic remission; M, male; NA, not applicable; Rec, recombinant.

*Defined as increase of the BCR-ABL load according to international scale to > 1% at any occasion after baseline.

†Patient 19 received pegylated IFN during induction and recombinant IFN during maintenance therapy.

effects. Of those, five had chronic diarrhea (grade 1, n = 4; grade 2, n = 1); eight had fluid retention (grade 1, n = 6, grade 2, n = 2), five had muscle cramps (grade 1, n = 2, grade 2, n = 2; grade 3, n = 1), three had grade 1 nausea, three had exanthema (grade 2, n = 2; grade 3, n = 1), two had liver toxicity (grade 2, n = 1; grade 3, n = 1), and one patient had grade 1 hair loss. One patient discontinued imatinib due to her wish for pregnancy. After imatinib discontinuation, initial IFN therapy was continued in all but one patient. One patient switched from pegylated to conventional IFN.

Assessment of Response and Relapse

Molecular response was assessed at baseline and every 2 to 3 months thereafter by determining the *BCR-ABL* to *ABL* mRNA transcript ratio isolated from the peripheral blood of the patients using quantitative reverse transcriptase polymerase chain reaction (Q-RT-PCR) and expressed according to the international scale (IS).[24] *BCR-ABL* transcripts at a level more than 0.1% to 1.0% IS were defined as minor molecular response; *BCR-ABL* transcript levels ≤ 0.1% IS indicated major molecular response (MMR); undetectable *BCR-ABL* by Q-RT-PCR and nested RT-PCR with at least 30,000 *ABL* transcripts per volume cDNA was referred to as complete molecular remission (CMR). Relapse was defined by an increase of the *BCR-ABL* load to greater than 1% (IS) at any single occasion.

Proteinase-3 Q-RT-PCR From Peripheral Blood mRNA

Blood samples were obtained after written informed consent and approval of the institutional review boards. Proteinase-3 transcript expression was quantified from total peripheral-blood leukocytes after mRNA extraction and cDNA synthesis[22] by Q-RT-PCR using primers MBN-1 5′-CTACATG GCCTCCCTGCAGAT-3′ and MBN-2 5′-TTGCGGCGAGGGACGAAA GTG-3′ and probes MBN-F 5′-TCTGAACAACTACGACGCGGAGAAC AA-F-3′ and MBN-Red 5′-LC-Red640-TGAACGACATTCTCCTCATCCA GCTGA-3′. Glucose-6-phosphate dehydrogenase (*G6PD*) transcripts served as internal control.[25] Final results were expressed as the ratio of proteinase 3/G6PD transcripts in percent.

Quantification of PR1-Specific CTLs in the Peripheral Blood

PR1-specific CTLs were labeled from peripheral-blood mononuclear cells (PBMCs) in 30 to 40 mL of heparinized peripheral blood as described previously.[21] PBMCs were incubated with 10 μL of CD8-PerCP Leu-2a (Becton Dickinson, Heidelberg, Germany) for 10 minutes at 22°C. Cells were washed twice with phosphate-buffered saline. Seven microliters of phycoerythrin-conjugated PR1-Pentamer (VLQELNVTV; Proimmune, Oxford, United Kingdom) and 3 μL of fluorescein isothiocyanate–labeled trash-antibody mix (CD4, CD19 [both mouse-antihuman, BD Pharmingen, San Diego, CA], CD14 [Immunotech, Marseille, France], CD56 [mouse-antihuman, BD Pharmigen]) was added, followed by incubation for 10 minutes on ice. Cells were washed, stained with 5 μL of 4′,6-diamidino-2′-phenylindole for dead-cell exclusion, and directly acquired on an LSRII (Becton Dickinson). Data were analyzed with FlowJo (version 8.8.4 for Macintosh, TreeStar, Ashland, OR).

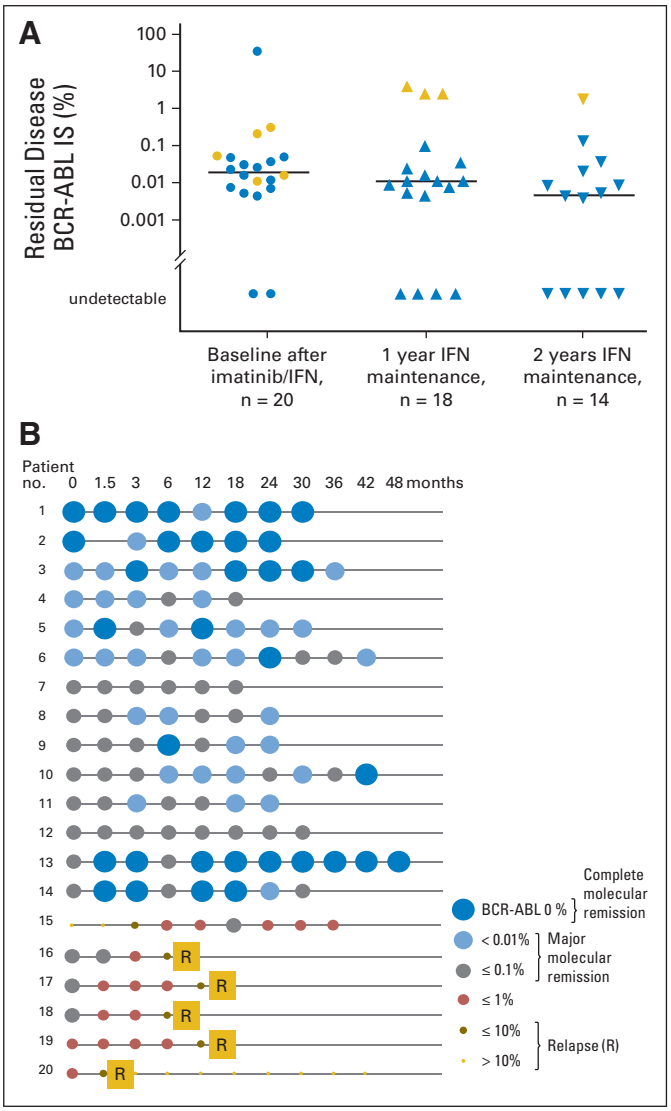

Fig 1. Molecular response to interferon alfa (IFN) maintenance therapy. (A) Residual BCR-ABL mRNA levels determined by quantitative reverse-transcriptase polymerase chain reaction (IS, international scale). Gold symbols: patients experiencing relapse. (B) Kinetics of residual *BCR-ABL* load after pausing imatinib. Two of five patients experiencing relapse were not in major molecular response at baseline.

RESULTS

Continuous Molecular Remissions With IFN Maintenance After Imatinib Discontinuation

Imatinib was discontinued in 20 patients with chronic-phase CML who had undergone a prior combination therapy with imatinib and IFN for more than 2 years (Table 1). At the time of stopping imatinib (ie, baseline), only one patient had not at least achieved CCR. Of the remaining 19 patients in CCR, 15 patients had achieved MMR and two had achieved CMR (Table 1; Fig 1A).

IFN maintenance therapy consisted of pegylated IFN (Pegasys; n = 16) at a dose of 90 to 180 μg/wk every 7 to 21 days or conventional IFN at 2 to 5 × 3 millon U per week (n = 4). During IFN maintenance therapy, molecular remission was monitored by Q-RT-PCR every 6 weeks to 3 months. The median *BCR-ABL* transcript level according to the IS declined in the entire cohort from 0.020% (range, 0% to 35%; n = 20) at baseline to 0.011% (range, 0% to 4%; n = 18) and 0.0048% (range, 0% to 1.8%; n = 14) 1 and 2 years after imatinib discontinuation, respectively (Table 1, Fig 1A). Thus after a median period of 2.4 years off imatinib (range, 0.5 to 4.0 years), 15 (75%) of 20 patients had either retained (n = 5) or improved (n = 10) the depth of their molecular remission by ≥ 1 log (IS) on at least one occasion (Fig 1B). Of note, the number of patients in CMR also increased with IFN monotherapy from two patients at baseline to five patients 2 years after discontinuation of imatinib. This documents a notable single-agent activity of IFN in the context of a prior imatinib/IFN combination therapy.

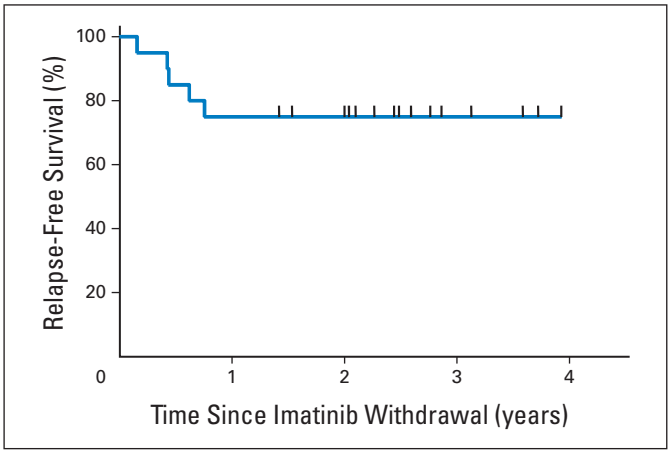

Fig 2. Relapse-free survival of patients treated with interferon alfa maintenance therapy. A total of five patients (25%) did not maintain their molecular remission from baseline and experienced relapse. Relapse was defined as an increase of *BCR-ABL* load to more than 1% (international scale) at any occasion. Tick marks indicate censored events.

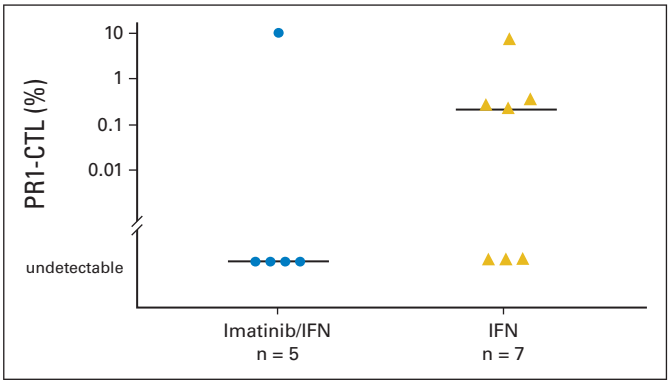

Fig 3. PR1-specific cytotoxic T cells (PR1-CTLs) quantitated by multicolor flow cytometry from up to 5 × 10^6 peripheral-blood mononuclear cells using PR1-specific pentamers. IFN, interferon alfa.

Relapses Under IFN Maintenance Occur Early and Can Be Rescued With Imatinib

Five patients (25%) revealed evidence of molecular relapse after imatinib discontinuation. Relapses occurred within a median time of 5.3 months (range, 1.9 to 9.1 months) after imatinib withdrawal (Fig 2). Of note, none of the five patients who experienced relapse had achieved a CMR before stopping imatinib, and two of them had reached less than MMR (Fig 1B). Hence depth of molecular remission at baseline may influence the potency of IFN in maintaining molecular remissions. The median *BCR-ABL* transcript level at relapse was 1.6% (range, 1.2% to 2.0%). In four patients experiencing relapse, recommencing imatinib monotherapy (400 mg/d) reestablished the prior major depth of molecular remission within a median of 7 months (range, 2 to 12 months). The fifth patient continued IFN monotherapy.

IFN Therapy Mediates Overexpression of Proteinase-3 and Expansion of PR1-CTL

IFN-, but not chemotherapy- or imatinib-induced remissions, were associated with the induction of PR1-CTL and proteinase-3 expression in monocytes.[20,21] Furthermore, IFN induces proteinase-3 expression in mononuclear cells,[21] and high proteinase-3 expression levels predict a good prognosis in CML.[26] We therefore prospectively assessed these putative markers of IFN response during the phase of imatinib/IFN combination and IFN maintenance therapy. PR1-specific CTLs were only detected in one of five assessable patients before imatinib withdrawal and in four of seven assessable patients during IFN maintenance therapy (Fig 3). PR1-CTL measurements during imatinib/IFN combination therapy and during IFN monotherapy were available for five patients. Patient 12 displayed PR1-CTL both during the imatinib/IFN combination therapy phase and after imatinib discontinuation. This was associated with a stable MMR throughout therapy (Fig 4A). Patient 17 was PR1-CTL negative during the combination therapy phase and did not develop PR1-CTL counts above the cutoff level of 0.02% with IFN monotherapy. This patient also failed to durably respond to IFN and experienced relapse approximately 9 months after imatinib withdrawal. In the most recent analysis, his PR1-CTL count was 0.033% (Fig 4B).

IFN monotherapy, when compared with the imatinib/IFN combination therapy, was associated with a significant increase in peripheral-blood proteinase-3-mRNA levels (Fig 5), indicating that imatinib limits circulating proteinase-3 antigen load.

DISCUSSION

Indefinite imatinib therapy is currently the recommended standard therapy in CML. However, permanent TKI intake raises concerns regarding the evolution of drug resistance,[10] long-term safety and tolerability,[11] compliance issues,[12] and costs.[13] The results presented here challenge the current view of permanent imatinib treatment as the unequivocal standard of care in CML. The conviction that ongoing ABL-TKI therapy is necessary to control CML is based primarily on in vivo evidence showing detectable *BCR-ABL* even after years of imatinib therapy[3,7,27] and furthermore by in vitro data demonstrating that CML precursors and stem cells may be inherently resistant to any form of ABL-TKI therapy.[4-6] Therefore, considerable efforts are being undertaken to identify means to target residual CML, with the ultimate goal to cure the disease and to discontinue imatinib.

So far, there is little evidence that imatinib can be successfully discontinued in patients who have achieved a CMR.[28-30] However, CMR is infrequent using sensitive RT-PCR methods, and the majority of patients may still experience relapse after CMR without imatinib.[8,28,29] Recent data from the French "Stop Imatinib" trial suggested that approximately 41% of the CMR cohort may eventually remain progression free after imatinib discontinuation, but follow-up is still short (21 months).[31]

With a median follow-up of 2.4 years in a small cohort of patients, we here provide evidence that the sequence of an imatinib/IFN-based first-line therapy followed by IFN maintenance results in sustained remissions in the majority of patients, irrespective of the prior achievement of CMR. This may have two major implications. By enabling discontinuation of imatinib, IFN maintenance addresses two major concerns of permanent ABL-TKI therapy: First, the evolution of drug resistance from a still-ambiguous reservoir of TKI-refractory, persisting CML stem cells and the issue of unknown long-term side effects. Second, IFN apparently targets residual BCR-ABL–positive cells, which may be insensitive to TKI therapy. This notion is at least

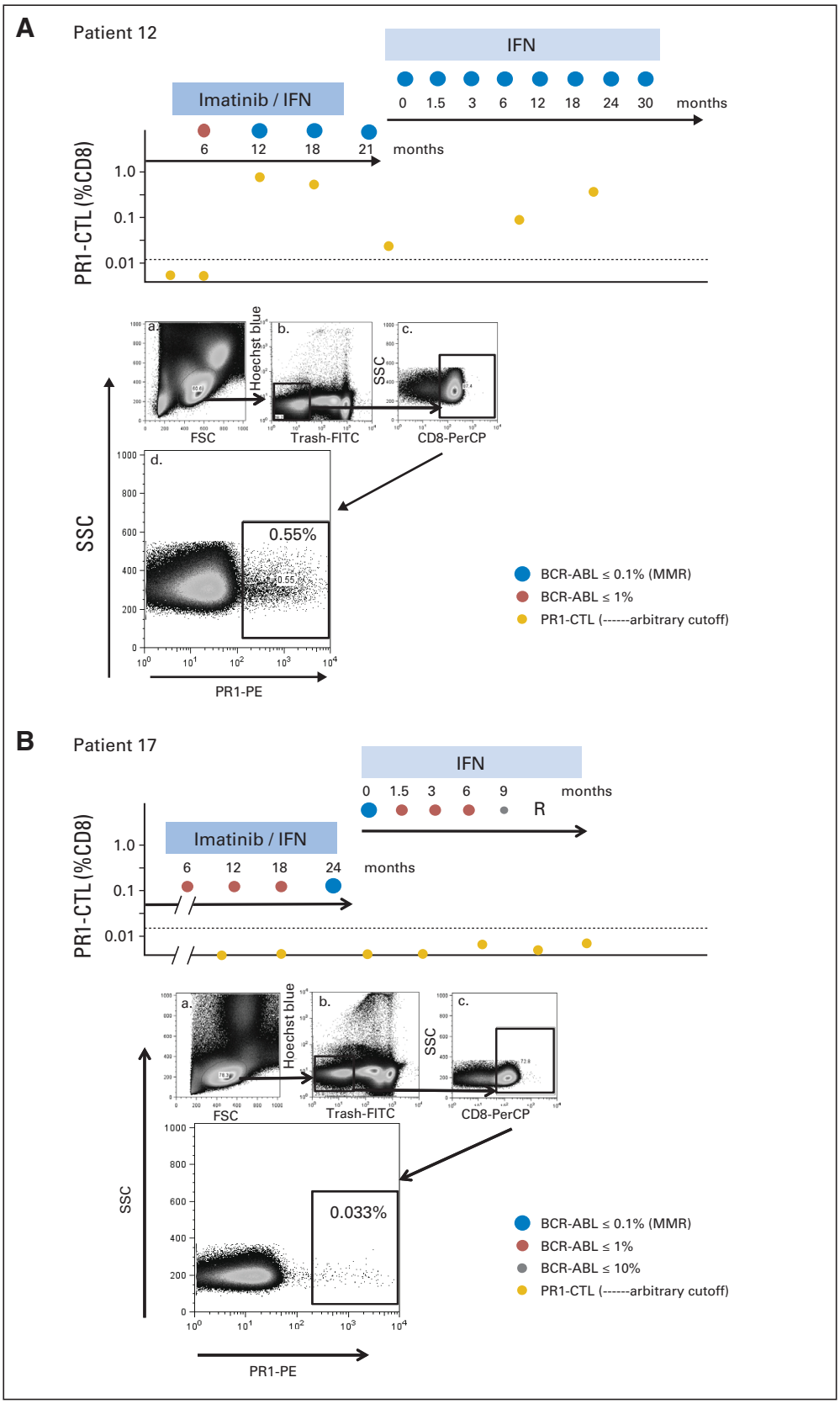

Fig 4. (A) Longitudinal analysis of PR1-specific cytotoxic T cells (PR1-CTL) in patient 12. Lower panel displays the triple gating strategy; (a) forward scatter (FSC) sideward scatter (SSC) on mononuclear fraction, (b) Hoechst blue, CD4-, 19-, 56-fluorescein isothiocyanate (FITC), (c) CD8-PerCP to CD8^{+} cells for PR1-phycoerythrin (PE)–pentamer binding. (B) PR1-CTL and molecular response in patient 17, who failed to maintain remission with interferon alfa (IFN) therapy.

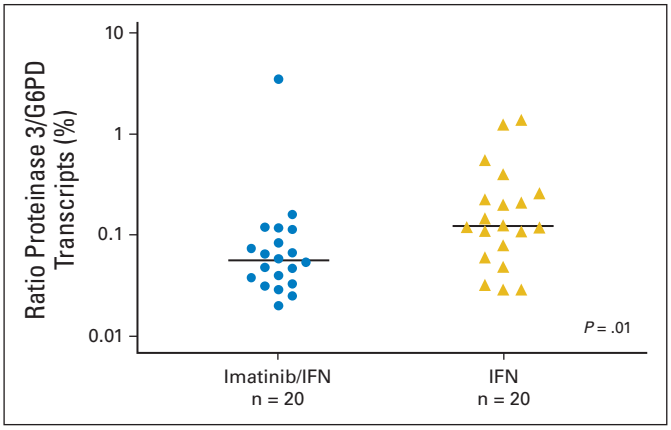

Fig 5. Quantitation of proteinase-3–mRNA expression during imatinib/interferon alfa (IFN) combination therapy before imatinib was discontinued versus IFN maintenance therapy (at least 3 months after imatinib withdrawal) by quantitative reverse-transcriptase polymerase chain reaction. *G6PD*, glucose-6-phosphate dehydrogenase transcripts used as control.

supported by the fact that IFN alone increased the depth of a molecular remission after imatinib was stopped.

Several reasons may account for this unprecedented treatment efficacy of IFN in the context of a sequential imatinib/IFN induction and IFN maintenance concept. On the one hand, the TKI-based vigorous CML debulking upfront may be an important cornerstone of subsequent IFN responsiveness, because it has been shown that high tumor burden induces T-cell senescence and apoptosis, thereby depleting antileukemic T-cell clones with the highest antileukemic potential.[32] On the other hand, imatinib was shown to inhibit T-cell activation[33-36] and the immunogenicity of CML cells via downregulating expression of BCR-ABL–associated self-antigens such as proteinase-3.[37,38] Thus only discontinuation of imatinib after debulking may hypothetically release the full immune-stimulatory potential of IFN.[18] Indeed, proteinase-3 mRNA levels and the frequencies of PR1-CTL further increased after patients had stopped imatinib (Figs 3 and 5). Circumstantial evidence for a direct inhibition of the expansion of PR1-CTL by imatinib was also prospectively documented in patient 3 and one patients with CML (patient 21) who was not part of the clinical study (Appendix Fig A1, online only). Recent observations also seem to imply that IFN may sensitize dormant stem cells to imatinib-induced apoptosis by inducing their cell cycle entry.[39] Altogether these data support the conclusion that a combined imatinib/IFN induction therapy could be of advantage compared with imatinib monotherapy and that IFN may overcome inhibitory effects of imatinib on the elicitation of antileukemic T-cell responses. However, the results presented here are only applicable to IFN maintenance treatment that follows an imatinib/IFN combination therapy. Whether IFN maintenance is as effective after imatinib monotherapy for induction cannot be inferred from this study. Of the five patients who lost remission during the IFN maintenance phase, all five experienced relapse within the first 9 months after discontinuation of imatinib, but regained their prior depth of molecular remission with resumption of imatinib. Of the three patients who had not obtained an MMR, two patients experienced relapse under IFN monotherapy, as opposed to only three IFN failures among the 17 patients who were in MMR at baseline. This suggests that lack of MMR increases the likelihood of IFN maintenance failure.

The toxicity profile of IFN during maintenance therapy was low, with no grade 3 or 4 adverse events (Table 1). This supposedly owes to the relatively low doses of IFN that were injected and the fact that 16 (80%) of 20 patients received pegylated IFN, with improved tolerability compared with standard IFN.

In summary, the concept of an upfront imatinib/IFN combination therapy aiming to obtain an MMR followed by IFN monotherapy to maintain this remission may become an attractive alternative to lifelong TKI therapy. Given the excellent long-term outcome of complete cytogenetic responders pausing IFN,[17,23] the induction/maintenance concept may even hold the promise to achieve durable disease control without any further therapy.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Appendix

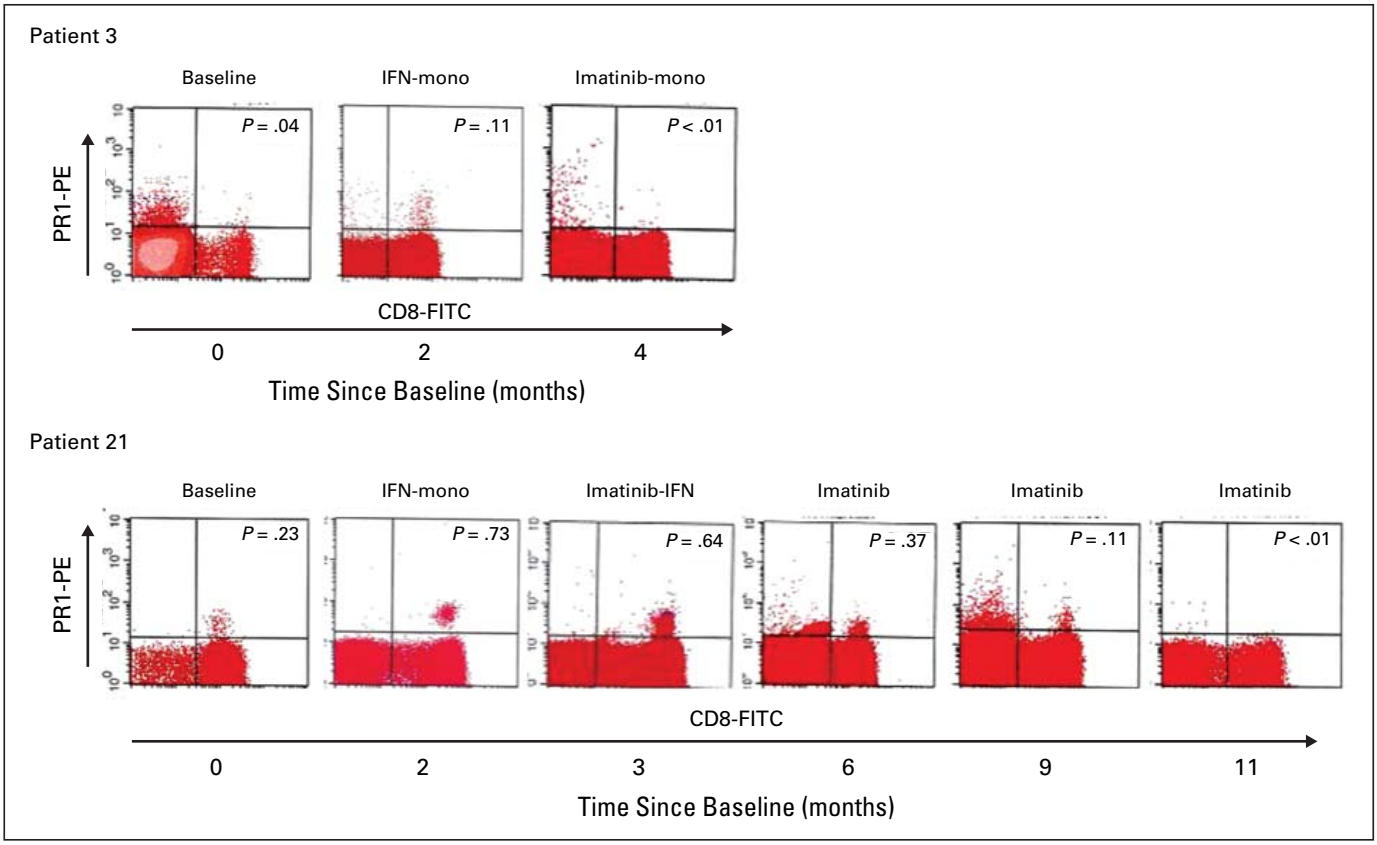

Fig A1. Treatment type–dependent regulation of PR1-specific cytotoxic T cells (PR1-CTL) frequency. Longitudinal assessment of $CD8^+$ PR1-CTL in the peripheral blood of patients with chronic myeloid leukemia at indicated time points using flow cytometry. Upper panel: Patient 3 received sequentially pegylated interferon alfa (IFN; 180 μg subcutaneously weekly) for 6 weeks followed by imatinib monotherapy (400 mg/day) for 6 weeks and then a combination of both. IFN rapidly stimulated PR1-CTL expansion, but after commencing imatinib, this population waned. Lower panel: As seen in patient 3, this patient (patient 21) was also treated up front with six injections of pegylated IFN (180 μg). This resulted in a rapid expansion of a distinct PR1-CTL population that was clearly inhibited with the introduction of imatinib. The patient was intolerant to IFN and discontinued IFN after 3 months. PR1-CTL became undetectable after 11 months, of which 7 were imatinib monotherapy. Exhaustion of PR1-CTL was not due to lack of antigenic stimulation (circulating proteinase 3), because the patient experienced a primary imatinib resistance and did not achieve any cytogenetic remission at this point. He was switched to nilotinib.

