Interferon-alpha in chronic myeloid leukemia revisited: A long-term retrospective study in Central and Northern Moravia

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Aims. We assessed the long-term outcome of consecutive patients in the chronic phase of chronic myeloid leukemia (CML) treated with interferon-alpha (INF-α) in Central and Northern Moravia between 1989 and 2006.
Methods. A retrospective study focused on the response, prognostic factors and side-effects of INF-α.
Results. 118 patients (67 males and 51 females, median age 50 years; range 18-71) were analyzed. The median follow-up was 82.6 months (12.4-212.6). Thirty-six patients (30.5%) achieved major cytogenetic response (CyR) in median of 18.3 months (3.7-47.3) and maintained it for a median of 64.0 months (7.0-176.0). Sixty-one patients treated with INF-α for more than 12 months had an overall survival (OS) of 137.0 months (95% CI 117.6-156.4). Eighteen (29.5%) achieved complete CyR (CCyR). 109 patients discontinued the treatment with INF-α because of hematologic or cytogenetic resistance in 53 (48.7%), progression of CML in 31 (28.4%) and intolerance to INF-α in 17 (15.6%) patients. The percentage of peripheral blasts, leukocyte count (>50x10^9/L), splenomegaly, anemia (Hgb≤110 g/L) and Sokal score had statistical impact on the OS in univariate assessment but only the Sokal score remained significant in multivariate analysis. Additional cytogenetic abnormalities at diagnosis were associated with poor prognosis.
Conclusions. In most patients, treatment with INF-α had to be stopped because of a failure to induce response, progression of CML or side-effects but nearly one third of patients treated at least for one year had a long-term benefit from INF-α. The best prognosis was associated with achievement of CCyR and negativity of BCR-ABL in nested RT-PCR.

Key words: chronic myeloid leukemia, interferon-alpha, cytogenetic response, BCR-ABL1, RT-PCR

Received: January 3, 2012; Accepted with revision: June 25, 2012; Available online: November 27, 2012
http://dx.doi.org/10.5507/bp.2012.068

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INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal myeloproliferative neoplasm characterized by acquisition of the Philadelphia (Ph) chromosome in stem cells and their progeny[1]. The aberrant chromosome results of a reciprocal translocation between the long arms of chromosomes 9 and 22 t(9;22)(q34;q11), giving rise to the fusion gene *BCR-ABL1*. This fusion gene encodes for a constitutively active tyrosine kinase which has become the major therapeutic target of CML treatment today[2,3]. Imatinib, a tyrosine kinase inhibitor (TKI) often exerts very rapid therapeutic response[4] that may be explained by targeting mature CML progenitors. However, responses achieved after imatinib therapy may not be durable[5] which could be a clinical correlation of CML stem cell resistance to TKIs. CML origin is supposed to arise from the transformed line of CD34+CD38- normal stem cells since these cells themselves and their progeny carry the *BCR-ABL1* fusion gene[6]. CML stem cells share many biological properties with their normal counterparts[7]. Hematopoietic stem cells are largely quiescent and express high levels of the multidrug resistance-1 gene[8] that might limit imatinib uptake[9]. In contrast to mature CML progenitors where BCR-ABL kinase is required for survival and self-renewal, in the case of CML stem cells, BCR-ABL seems to play only a subtle role and is not indispensable for their self-renewal[7,10-12].

Interferon-alpha (INF-α) was one of the main treatment options before the discovery of TKIs. Reports of complete cytogenetic response (CCyR) and complete molecular response (CMR) achieved after INF-α therapy have been published[13]. Achieving CCyR and CMR was proved to be associated with excellent long-term prognosis[14]. Some patients maintained their CCyR even after discontinuation of INF-α and remained without any specific treatment[13]. In comparison with imatinib, CCyR after INF-α was achieved in a median of 19 months (6 months with imatinib) and was long-lasting[15]. There is laboratory[16] and clinical evidence showing that INF-α is effective in targeting quiescent CML stem cells insensitive to TKIs. Angstreich et al. tested *in vitro* sensitivity of primitive and differentiated CML progenitors to imatinib and INF-α proving that imatinib is more toxic to CML CFU-GM progenitors whereas INF-α had significantly greater activity against primitive Ph-positive progenitors[17]. INF-α exerts its wide range of effect via multiple pathways

involved in cytokine gene expression, cell cycle regulation, differentiation, proliferation and apoptosis[18]. It also stimulates autologous cytotoxic T lymphocytes to specifically recognize BCR-ABL or BCR-ABL-dependent antigens like proteinase-3 (ref.[19]). It was shown that INF-α, but not imatinib, triggers PR1-specific cytotoxic T lymphocyte responses[20], a fact that is considered to be associated with responsiveness to INF-α in CML (ref.[20-22]).

In our long-term retrospective study, we evaluated the outcome of all consecutive CML patients treated in the Northern and Central Moravia regions with INF-α alone or in combination with cytosine arabinoside (Ara-C) or hydroxyurea, with special attention to treatment response, side-effects and assessment of prognostic factors.

MATERIALS AND METHODS

Patients and treatment

All consecutive patients with chronic phase CML diagnosed and treated with INF-α at the Department of Hemato-Oncology of the Faculty of Medicine and Dentistry, Palacky University in Olomouc in cooperation with regional hematologists between 1989 and 2006 were included in the analysis. Patients participating in clinical studies performed during the study period gave informed consent approved by the local Ethics Committee. Transplanted patients and patients whose data were collected in the EBMT and CAMELIA databases consented to data collection.

Diagnosis of CML was based on cytogenetic assessment with confirmation of the Ph chromosome and/or demonstration of the *BCR-ABL1* fusion gene using nested RT-PCR or fluorescence *in situ* hybridization (FISH). Cytogenetic examinations were performed according to the standard procedure from bone marrow cells at the time of starting INF-α therapy and then once every six months or in cases of suspected progression. At least 20 mitoses were analyzed. Interphase FISH with LSI BCR-ABL ES and/or LSI BCR-ABL Dual Fusion probes (Abbott-Vysis, Downers Grove, USA) was performed in cases of culture failure. In case of additional cytogenetic abnormalities (ACyA), FISH with centromeric (Abbott-Vysis) and/or painting probes (Cambio Ltd., Cambridge, UK) was performed. Molecular response was assessed by nested RT-PCR (ref.[23]) and from 2000 also by real-time quantitative reverse-transcriptase polymerase chain reactions (RQ-PCR). The detailed method for RQ-PCR was described previously[24]. CML phases were defined according to a study by Cortes et al.[25]. Sokal and Hasford scores were calculated in patients at diagnosis before introduction of any treatment, according to previously published formulas[26,27].

Patients were evaluated for hematologic and cytogenetic (CyR) responses and their median duration met the ELN criteria with the exception of the first bone marrow punction done in 6 months instead of 3 months[28]. Complete hematologic response (CHR) was defined as platelet count less than $450x10^9$/L, white blood cell count (WBC) less than $10x10^9$/L, no immature granulocytes, less than 5% of basophils, and no palpable spleen. Cytogenetic response was defined as complete (no Ph chromosome-positive cells in at least 20 metaphases obtained from bone marrow aspirate), partial (1-35% of Ph chromosome-positive cells), minor (36-65% of Ph chromosome-positive cells), or minimal (66-95% of Ph chromosome-positive cells). In cases of cytogenetic culture failure, 1% of cells positive at interphase FISH examination was used as the cut-off for CCyR. We also studied the median time in which patients achieved their first and maximal CyR. Negativity of BCR-ABL was confirmed in all cases exclusively by nested RT-PCR.

Roferon (Roche, Basel, Switzerland) or Intron-A (Schering-Plough, Kenilworth, USA) were used for the treatment. INF-α was applied in a single daily subcutaneous injection usually in the evening with paracetamol used for prevention and treatment of flu-like syndrome at the beginning of the therapy. The treatment was initiated after cytoreduction with hydroxyurea with a target level of WCB≤$20x10^9$/L. The initial dose of 1.5 or 3 MU was increased depending on the patient's tolerance to 9 or 10 MU daily within one month. In patients who did not achieve CHR with this dose and in cases with WBC>$5x10^9$/L, hydroxyurea or subcutaneous Ara-C at a dose of 10 to 20 mg daily or oral Ara-C 40 mg daily was added in order to achieve WBC=4-$2x10^9$/L with absolute neutrophil count over $1.0x10^9$/L. In cases with more prominent neutropenia or thrombocytopenia less than $50x10^9$/L, the dose of INF-α was reduced appropriately. The lowest dosage used in case of hematologic toxicity was 3 MU three times weekly. In patients who achieved negativity of BCR-ABL in nested RT-PCR with at least two-year duration dose of INF-α was reduced very slowly during one to two years. For the purpose of autologous hematopoietic stem cell transplantation (HSCT), peripheral stem cells were collected after priming with chemotherapy and G-CSF. The priming was timed early after diagnosis after successful cytoreduction. The graft was frozen using the ICE-CUBE programmed freezer after addition of albumin and 10% dimethyl sulphoxide. Oral high-dose busulfan was used for conditioning in all transplanted patients. Data on all transplantations were submitted to the EBMT database and some of the patients were included in the EBMT CML 99 study.

Data analysis and statistics

Since the whole group of 118 patients comprised some who were switched to other treatment options due to INF-α failure or adverse effects or were treated with INF-α for less than 12 months, a group of patients treated with INF-α for more than 12 months was analyzed separately. Patients switched to TKIs during the follow-up were censored at the time of TKI initiation in the overall survival (OS) estimation. Patients in whom allogeneic HSCT was performed were censored for analysis of OS at the time of HSCT. The OS was calculated from the first dose of INF-α and from the first CyR achieved. The prognostic impact of splenomegaly, initial hemoglobin levels, leukocyte and platelet counts, percentage of peripheral blasts, eosinophils and basophils, ACyA, age and influence of

Sokal and Hasford (Euro) scores on the OS of the whole cohort and patients treated with INF-α for more than one year was evaluated.

The log-rank test, Mann-Whitney U test and Cox regression analysis were used in statistical evaluation[29]. The OS was calculated using the Kaplan-Meier method, with 5% level being considered as statistically significant[30]. Landmark analysis was performed to calculate the OS in patients who achieved CCyR that lasted over 12 months.

RESULTS

The study group comprised 118 consecutive adult Ph-positive CML patients in the chronic phase of the disease at the time of diagnosis. Main characteristics of all 118 patients at the time of diagnosis are listed in Table 1. In 11 patients, ACyA were detected and variant Ph chromosome was detected in 4 patients. The median time from diagnosis to treatment initiation was 1.0 month (range 0-43.0 months). Patients were treated with INF-α for a median period of 12.0 months (0.3-192.0). The median overall follow-up was 82.6 months (12.4-212.6). Patients were treated with an average INF-α dose of 9 MU (1.5-10.0) daily. Sixty-six patients (55.9%) were treated with INF-α in combination with hydroxyurea, thirty-two patients (27.0%) in combination with low-dose Ara-C and seven patients (5.9%) underwent autologous HSCT. After INF-α discontinuation, seventy-seven patients (65.3%) were switched to therapy with TKIs. Twelve patients (10.0%) underwent allogeneic HSCT. The treatment regimens are summarized in Table 2.

General overview of achieved responses and reasons for INF- α discontinuation

As shown in Table 3, of all 118 patients treated with INF-α, seventy-three (61.9%) achieved CHR in a median of 3 months (1.0-14.0), calculated from the first dose of INF-α. The intention-to-treat analysis (118 patients) showed that 41 patients (34.8%) achieved their maximal CyR in a median of 12 months (0.3-47.0) with a median duration of 23 months (1.0-176.0). The best CyR was partial (PCyR) in 18 patients (15.3%) and complete in 18 patients (15.3%), achieved in a median time of 18.3 months (3.7-47.3) and maintained for a median of 64.0 months (7.0-176.0). In 12 patients (10%), CyR was not evaluated. The mean OS in all 118 was 145.3 months (95% CI 130.9-159.8), as shown in Fig.1.

A total of 109 patients discontinued treatment with INF-α (Table 4). The main reasons were a lack of hemato-

Table 1. Characteristics of 118 patients treated with INF-α and INF-α combined regimens.

	No. (%)	Median (range)
Age, years	118	50 (18-71)
Male	67 (56.8%)	48 (18-71)
Female	51 (43.2%)	53 (21-71)
Spleen (palpable, cm)	66 (56%)	1 (0-20)
Peripheral blood		
Hemoglobin level ≤110 g/L	31 (26.3%)	126 (70-161)
Leukocyte count x 10^9/L		86 (2-777)
Platelet count x 10^9/L		311 (68-2013)
Basophils %		4 (0-19)
Eosinophils %		2 (0-13)
Promyelocytes %		1 (0-15)
Blasts %		1 (0-14)
Hasford (Euro) score		941.5 (41.3-2065)
Low risk	45 (38%)	
Intermediate risk	50 (43%)	
High risk	22 (19%)	
Sokal score		0.87 (0.51-3.1)
Low risk	46 (39%)	
Intermediate risk	46 (39%)	
High risk	25 (21%)	
Bone marrow		
ACyA	11 (9%)	

ACyA, Aditional cytogenetic abnormalities.

logic or cytogenetic response in 53 (48.7%) patients, progression of CML that occurred after temporal response in 23 (21.1%) and intolerance to INF-α in 17 (15.6%) patients. Eight patients finally progressed to the accelerated or blastic phase of CML. In 7 patients, the treatment with INF-α was stopped after achievement of BCR-ABL negativity in RT-PCR.

Eleven patients had ACyA at the time of diagnosis (2 had trisomy 8, one inversion 17q, one deletion of chromosome Y, 3 had additional translocations and 4 other deletions). In these patients, INF-α was discontinued due to hematologic (3 patients) or cytogenetic resistance (1 patient), cytogenetic progression (2 patients) and evolution of the blastic phase (1 patient). In three patients, INF-α treatment was discontinued due to intolerance to the drug. In 6 patients, no CyR was achieved. Five patients were switched to imatinib. As of the date of this report,, 7 patients with ACyA at diagnosis have died, 3 patients are alive on imatinib and 1 patient with ACyA (trisomy 8) continues INF-α treatment after having achieved negativity of BCR-ABL in nested RT-PCR. Four additional patients had variant Ph chromosome at the time of diagnosis. One of them died in the blastic phase of CML while the others were switched to imatinib.

Table 2. Summary of the treatment used during the follow-up.

Therapy	No.	%
INF-α	13	11
INF-α in combination with hydroxyurea	66	55.9
INF-α in combination with Ara-C	32	27.1
INF-α in combination with autologous HSCT	7	5.9
Therapy after INF-α discontinuation		
Allogeneic HSCT	12	10.2
Therapy with TKIs	77	65.3

Ara-C, Cytosine arabinoside; HSCT, Hematopoietic stem cell transplantation; INF-α, Interferon-alpha; TKI, Tyrosine kinase inhibitor.

Table 3. Summary of treatment response assessed in all 118 patients treated with INF-α.

118 patients	No.	%	Median time from the first INF-α dose (months)	Median duration (months)
CHR	73	61.9	3 (1-14)	
Best CyR	41	34.8	12 (0.3-47)	23 (1-176)
Complete CyR	18	15.3	18.3 (3.7-47.3)	64 (7-176)
Partial CyR	18	15.3		
Minor CyR	3	2.5		
Minimal CyR	2	1.7		
CyR not evaluated	12	10.1		

CHR, Complete heresponse; CyR, Cytogenetic response.

Table 4. Reasons for INF-α discontinuation in 109 patients.

Reasons for INF-α discontinuation	No.	(%)
HRES	26	23.9
CRES	27	24.8
HPROG	4	3.7
CPROG	19	17.4
Intolerance	17	15.6
AP	2	1.8
BP	6	5.5
HSCT	2	1.8
CMR	7	6.4

AP, Accelerated phase; BP, Blastic phase; CMR, Complete molecular response; CPROG, Cytogenetic progression; CRES, Cytogenetic resistance; HPROG, Hematologic progression; HRES, Hematologic resistance; HSCT, Hematopoietic stem cell transplantation; INF-α, Interferon-alpha.

All factors mentioned in the Materials and Methods section were evaluated for their impact on prognosis (namely the overall survival) of the whole patient cohort. The percentage of peripheral blasts, leukocyte count (WBC>50x10^9/L), splenomegaly, anemia (Hgb ≤110 g/L) and Sokal score had a statistical impact on the OS in univariate assessment but only the Sokal score remained significant in multivariate analysis. Each increase in Sokal risk score in different patients at the time of diagnosis (from low to intermediate and to high) was associated with a 2.03-fold (95% CI 1.31-3.16) increased risk of death (*P*=0.002).

INF-α was discontinued due to intolerance (17 patients; 27.9%), treatment failure (23 patients; 37.7%) or sustained negativity of BCR-ABL in nested RT-PCR (7 patients; 11.5%). A detailed overview of reasons for discontinuation with numbers of patients is shown in Table 4. The most common adverse effects noted were flu-like syndrome, anorexia with significant weight loss, insomnia, allergic exanthema and liver toxicity. The most severe side-effects were rare and involved nervous system disorders including Parkinson syndrome (1 patient), polyradiculitis (1 patient) and depression. One patient committed suicide as a consequence of depression probably caused by INF-α despite anti-depression treatment managed by a psychiatrist.

Survival and prognostic factors for patients treated with INF- α

Sixty-one patients treated with INF-α for a period of time longer than 12 months were eligible for estimation of the OS and evaluation of prognostic factors. Patients who started INF-α after 2000 and those who were treated for less than 12 months were excluded to prevent statistical bias from too short treatment that could modify response evaluation. The mean OS calculated from the first dose of INF-α and from the first CyR achieved was 137.0 months (95% CI 117.6-156.4) and 139.6 months (95% CI 120.5-158.8) with a probability of 5-year survival of 73% and 83%, respectively (Fig. 2 and 3). The median OS was not achieved. The initial Sokal score was confirmed to have significant influence (*P*=0.014) on the estimated mean OS with 153.5 months (95% CI 127.0-180.0), 130.0 months (95% CI 101.2-158.8), and 68.6 months (95% CI 41.2-96.0) for low-risk, intermediate-risk and high-risk, respectively (Fig. 4). However, no significant difference in the OS was observed using Hasford score. Hemoglobin level at the time of diagnosis with 110 g/L as the cut-off was proved to be significant as a prognostic factor (*P*<0.0001) with the mean OS of 84.5 months (95% CI 49.8-119.2) in patients with Hgb<110 g/L and 161.9 months (95% CI 140.9-183.0) in patients with Hgb>110 g/L (Fig. 5). Initial splenomegaly was also confirmed to have a prognostic impact (*P*=0.011) on the mean OS with 150.0 months (95% CI 134.3-166.1) and 136.0 months (95% CI 114.7-156.3) for patients with normal spleen size and any spleen enlargement, respectively. In univariate analysis, the Sokal risk score, spleen size and hemoglobin level (110 g/L as the cut-off) all proved to be independent factors for an increased risk of death. In Cox multivariate regression analysis, Sokal risk score remained the only independent prognostic factor. Each increase in Sokal risk score (from low to intermediate and to high) was associated with 3.21-fold (95% CI 1.67-6.15) increased risk of death (*P*=0.0004). The initial platelet count, WBC, percentage of basophils, eosinophils, age and sex showed no significance in multivariate analysis.

In a subgroup of 61 patients treated with INF-α for more than 12 months, CyR was analyzed and expressed as cumulative probability in Fig. 6. Achieving first CyR irrespective of its quality was expected in a median of 9.0 months (95% CI 4.6-13.5). Clinically important responses (PCyR and CCyR) were expected to be achieved in a median of 20.0 months (95% CI 11.7-28.4). CCyR

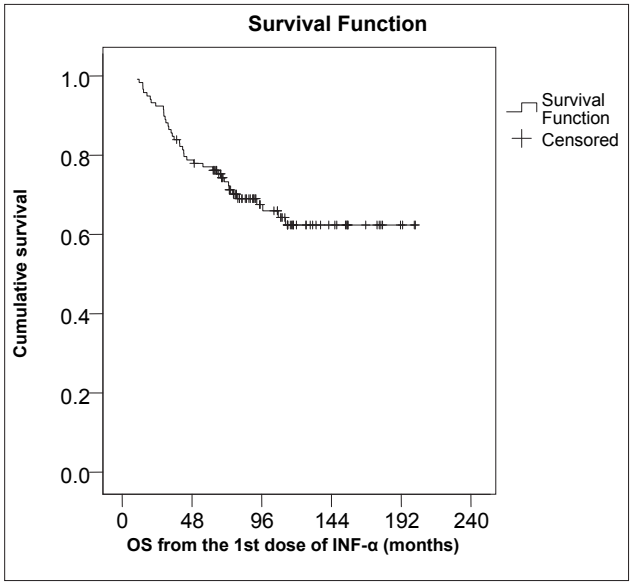

Fig. 1. Mean OS of all 118 patients treated with INF-α calculated from the 1st INF-α dose was estimated at 145.3 months (95% CI 130.9-159.8).

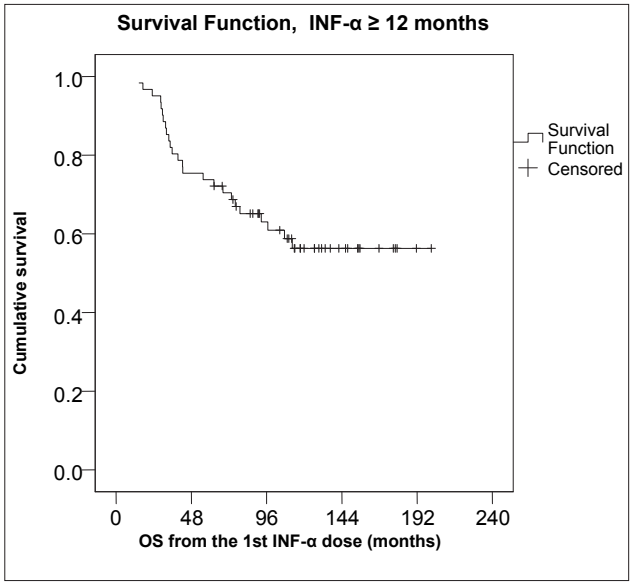

Fig. 2. Mean OS of 61 patients treated with INF-α for more than 12 months calculated from the 1st INF-α dose was estimated at 137 months (95% CI 117.6-156.4).

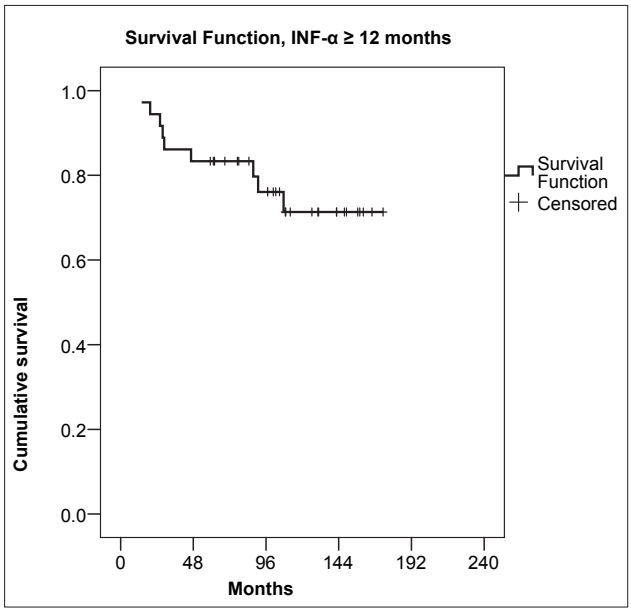

Fig. 3. Mean OS of 61 patients treated with INF-α for more than 12 months calculated from the 1st CyR was estimated at 139.6 months (95% CI 120.5-158.8).

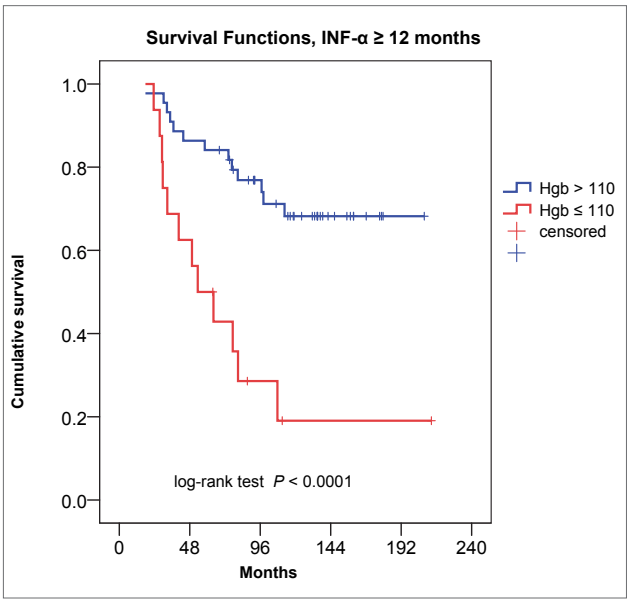

Fig. 5. OS from date of diagnosis estimated according to the hemoglobin cut-off level 110 g/L with mean OS of 84.5 months (95% CI 49.8-119.2) and 161.9 months (95% CI 140.9-183).

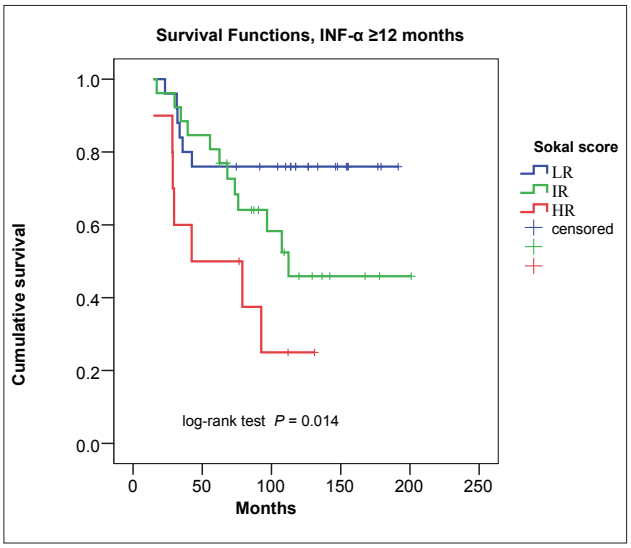

Fig. 4. OS estimated according to Sokal score (LR, IR, HR) at 153.5 months (95% CI 127-180), 130 months (95% CI 101.2-158.8) and 68.6 months (95% CI 41.2-96), respectively.

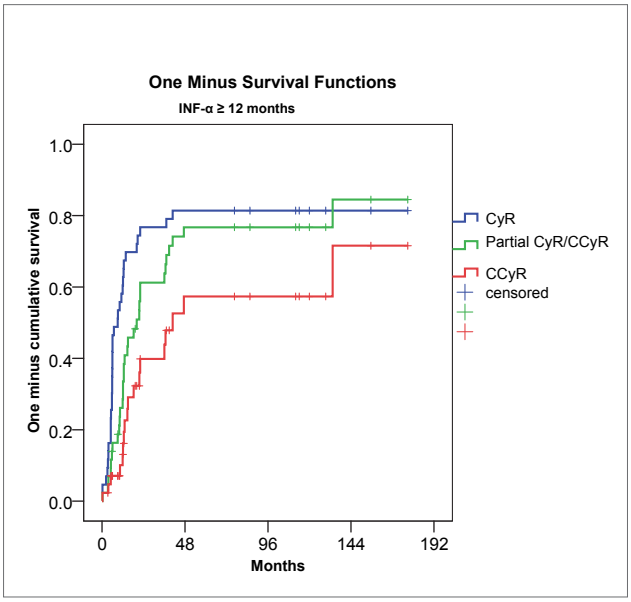

Fig. 6. Cumulative probability of achievement of the first CyR in estimated time from the first INF-α dose. The upper curve shows cumulative probability of achievement of the first CyR regardless of its quality; the median time to CyR is 9 months (95% CI 4.6-13.5). The middle curve shows the cumulative probability of achievement of the first PCyR or CCyR whichever came first; the median time to the event is 20 months (95% CO 11.7-28.4). The lower curve shows the cumulative probability of achievement of the first CCyR; the median time to CCyR has been estimated at 40.8 months (95% CI 25.1-56.6).

achievement was estimated in a median of 40.8 months (95% CI 25.1-56.6) (Fig. 6).

Complete cytogenetic responders

Eighteen patients achieved their CCyR as a result of INF-α therapy in a median of 18.3 months (3.7-47.3) and maintained their response for a median of 64 months (7-176). Patients were treated with INF-α for a median of 61.0 months. The majority of complete responders (67%) were classified as low risk according to Sokal score while 6 patients had an intermediate risk. No ACyA were detected at diagnosis in any of complete cytogenetic responders except one patient who is still on INF-α therapy in sustained negativity of BCR-ABL in RT-PCR. Landmark

analysis (Fig. 7) in complete responders who maintained their response for at least 12 months (16 patients) estimated mean OS of 175.9 months (95% CI 144.4-207.7) with a probability of approximate survival of 87% at 5 years. Nine complete responders achieved negativity of BCR-ABL in RT-PCR and six of them discontinued INF-α due to prolonged RT-PCR negativity. Of the patients who achieved only CCyR without achievement of RT-PCR negativity, 6 patients lost their CCyR during follow-up and were switched to treatment with TKIs. One patient continues on INF-α and is still in CCyR. One patient died without the loss of CCyR from suicide due to severe depression as mentioned above. INF-α therapy was a highly probable cause of depression in this case. One patient died in a sudden blastic transformation of CML 21 months after achievement of CCyR.

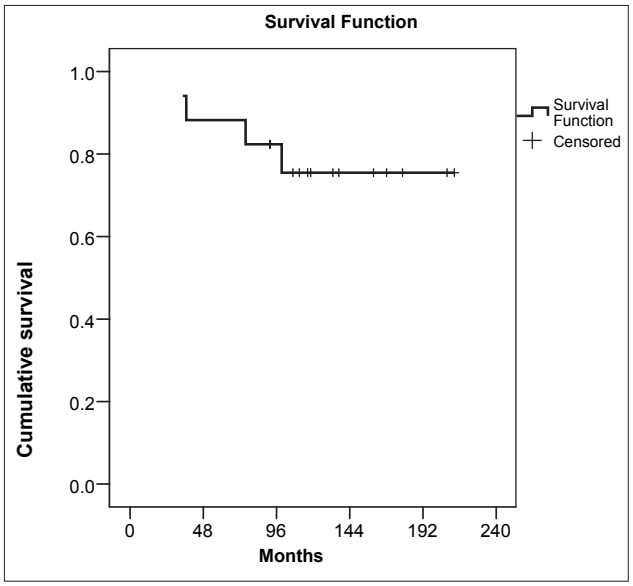

Fig. 7. Landmark analysis in complete cytogenetic responders who sustained CCyR for more than 12 months with the OS estimated at 175.9 months (95% CI 144.2-207.7).

DISCUSSION

Despite various severe adverse effects related to INF-α therapy leading to INF-α discontinuation and numerous cases of treatment failure due to primary INF-α resistance or disease progression, there is a group of patients that may benefit from this treatment option. Our results correspond with those from the literature[13-15,31] proving that complete cytogenetic and especially molecular responses achieved on INF-α were rather long-lasting. Moreover, achievement of negativity of BCR-ABL in RT-PCR by INF-α enabled cessation of the treatment in a larger proportion of patients as compared than those on imatinib. As mentioned in the Introduction, this may be explained by the immunomodulatory effects of INF-α and its capability to target CML stem cells.

In our study, the proportion of patients who achieved BCR-ABL negativity in RT-PCR was 7.6%, one of the highest of single-center intention-to-treat analyses published. Kantarjian et al. published the only single center analysis with 512 patients treated with INF-α-based regimens where 20 patients (3.9%) were defined as complete molecular responders in at least 2 negative RT-PCR tests without a subsequent positive result[14]. They concluded that achievement of CCyR and CMR was associated with excellent long-term event-free survival of the patients. In our study, a higher probability of CMR achievement was found and, unlike the Houston study, we did not observe isolated negativity results in nested RT-PCR testing. All our patients who achieved RT-PCR negativity never experienced recurrence of the disease. On the other hand, our analysis showed that despite a significant prognostic impact of CCyR on the OS, achievement of CCyR without BCR-ABL negativity in RT-PCR (CMR) does not automatically lead to "operational cure".

Although we excluded all patients treated with INF-α for a short period of time after the year 2000 from the final analysis, the 5-year OS achieved in our study group (70%) with subsequent TKI therapy was comparable to results in the literature. CML patients treated with INF-α, HSCT or TKIs in Germany from 1997-2001 achieved a 5-year OS of 71% (ref.[32]). The median OS was not achieved in our study group. However, more than a half of our patients treated with INF-α for more than 12 months finally failed to respond to the therapy and were switched to TKIs later on. Thus, these patients were censored in OS calculation at the time of TKI initiation. However, in our cohort of patients, achieving CCyR was confirmed to be associated with better prognosis and long-term survival with a 5-year OS of approximately 87%.

In univariate analysis, the percentage of peripheral blasts, leukocyte number (WBC>50x10^9/L), splenomegaly, anemia (Hgb≤110 g/L) and Sokal score had a statistical impact on the OS for the whole group, and Sokal score, splenomegaly and hemoglobin level lower than 110 g/L for patients treated for more than one year. Only the Sokal score remained independently significant in multivariate analysis in both cohorts of patients. Neither age, nor white cell count or platelet count, or percentage of eosinophils and basophils had significantly impact on OS. In addition, we confirmed the poor prognosis of patients with ACyA at diagnosis treated with INF-α. On the other hand, we identified some features in blood count associated with achievement of molecular response and negativity of BCR-ABL in RT-PCR (ref.[33]). However, this analysis should be viewed cautiously because of the low numbers of patients analyzed.

Although INF-α is no longer the front-line treatment option in CML management, it is still a possible alternative in patients with a good initial response to this treatment or those in whom therapy with TKIs is contraindicated. Because of its unique immunological effects and ability to target CML stem cells it has become an object of interest in novel clinical trials combining INF-α with TKIs (ref.[34-36]). Combination regimens showed promising results in a German study using imatinib with INF-α as induction with subsequent INF-α alone as maintenance therapy inducing a progressive decline in *BCR-ABL1* transcript levels and increase in CMR during the maintenance

phase after imatinib discontinuation[37]. In this study, only 25% of patients experienced molecular relapse during a median of 5.3 months. In the French stop-imatinib STIM study, however, 61% of patients relapsed during the first 6 months after imatinib discontinuation[38]. According to the SPIRIT study, a phase III randomized trial organized by the French CML Group, combination regimen containing imatinib with PEG INF-α-2a rendered the highest rate of major molecular response (62%) compared with standard (41%) or high-dose imatinib (52%) (ref.[36]). However, these results were not confirmed by the study carried out by authors from MD Anderson where no difference in major molecular response rates was observed in patients treated with high-dose imatinib only in comparison to those treated with the combination regimen containing high-dose imatinib, PEG INF-α-2b and granulocyte-macrophage colony-stimulating factor[34].

There are very little data on immunological markers that could predict the therapeutic effect of INF-α. So far, it has been reported that the presence of PR1-specific cytotoxic lymphocytes is associated with responsiveness to INF-α (ref.[19]). Our collaboration with Finnish authors revealed a unique plasma cytokine profile and increase in NK-cells and in T-lymphocyte subpopulation in patients able to discontinue INF-α (ref.[39]). However, the predictive role of this specific profile needs to be confirmed in prospective studies in order to assess its actual impact on INF-α treatment response and to help select patients with a higher probability of achieving CMR with INF-α therapy.

CONCLUSIONS

To summarize, our single-center retrospective study which analyzed the results of INF-α therapy corresponds with the reported literature and contributes to the ongoing quest for different ways towards permanent cure of CML. We found 15.3% and 7.6% achievement rates of CCyR and BCR-ABL negativity in RT-PCR, respectively, after INF-α in a group of 118 consecutive patients with chronic phase CML treated in a defined region in an intention-to-treat retrospective analysis. Unlike patients with only CCyR, the majority of whom lost CCyR despite continuing INF-α therapy and later required imatinib, patients who achieved BCR-ABL negativity in nested RT-PCR had excellent long-term outcome.

ABBREVIATIONS

ACyA, Aditional cytogenetic abnormalities; AP, Accelerated phase; Ara-C, Cytosine arabinoside; BP, Blastic phase; CCyR, Complete cytogenetic response; CHR, Complete hematologic response; CML, Chronic myeloid leukemia; CMR, Complete molecular response; CPROG, Cytogenetic progression; CRES, Cytogenetic resistance; CyR, Cytogenetic response; FISH, Fluorescence in situ hybridization; Hgb, Hemoglobin; HPROG, Hematologic progression; HR, High risk; HRES, Hematologic resistance; HSCT, Hematopoietic stem cell transplantation; IM, Intermediate risk; INF-α, Interferon-alpha; LR, Low risk; OS, Overall survival; PCyR, Partial cytogenetic response; Ph, Philadelphia chromosome; RQ-PCR, Real-time quantitative reverse-transcriptase polymerase chain reactions; RT-PCR, Reverse transcriptase-polymerase chain reaction; TKI, Tyrosine kinase inhibitor; WBC, White blood cell count.

ACKNOWLEDGEMENT

We acknowledge the contribution of all participating physicians: Department of Hemato-Oncology, University Hospital Olomouc: Marian Heczko, Antonin Hlusi, Jaromir Hubacek, Tomas Papajik, Vit Prochazka, Jana Prochazkova, Ludek Raida, Sarka Rozmanova, Ivana Skoumalova, Premysl Slezak, Ivana Sulovska, Romana Szotkowska, Tomas Szotkowski, Renata Urbanova, Jana Vondrakova.

Regional hematologists: Milan Bily, Sarka Blahutova, Martin Brejcha, Boris Bubenik, Michaela Cerna, Vera Farbiakova, Jaromir Gumulec, Vladimira Heinzova, Zdenka Hrebackova, Daniel Janek, Jirina Kovacova, Ivana Krajsova, Miluse Kubeckova, Blahoslav Kuca, Lenka Langrova, Jana Laska, Zenon Lasota, Eva Lautnerova, Tamara Lhotanova, Milena Lukesova, Milan Matuska, Marie Micanikova, Libuse Novosadova, Milan Pacejka, Jan Ptak, Stefan Repovsky, Pavel Schlemmer, Karel Srovnalik, David Starostka, Jiri Slezar, Elena Sumna, Milada Svarna, Marie Urbankova, Milena Vranova, Marek Wrobel, Jarmila Zivna.

The work was supported by grants MSM 6198959223, MSM 6198959205 and LFUP 2012-007 (Ministry of Education, Youth and Sports, the Czech Republic). We are grateful to Mgr. Pavel Kurfürst for editorial help with the manuscript.

CONFLICT OF INTEREST STATEMENT

Author's conflict of interest disclosure: *None declared.*

REFERENCES

1. Fialkow PJ, Gartler SM, Yoshida A. Clonal origin of chronic myelocytic leukemia in man. Proc Natl Acad Sci U S A 1967;58:1468-71.
2. Faderl S, Talpaz M, Estrov Z, Kantarjian HM. Chronic myelogenous leukemia: biology and therapy. Ann Intern Med 1999;131:207-19.
3. Druker BJ. Translation of the Philadelphia chromosome into therapy for CML. Blood 2008;112:4808-17.
4. Kantarjian HM, O'Brien S, Cortes J, Giles FJ, Rios MB, Shan J, Faderl S, Garcia-Manero G, Ferrajoli A, Verstovsek S, Wierda W, Keating M, Talpaz M. Imatinib mesylate therapy improves survival in patients with newly diagnosed Philadelphia chromosome-positive chronic myelogenous leukemia in the chronic phase: comparison with historic data. Cancer 2003;98:2636-42.
5. Cortes J, O'Brien S, Kantarjian H. Discontinuation of imatinib therapy after achieving a molecular response. Blood 2004;104:2204-5.
6. Fialkow PJ, Denman AM, Jacobson RJ, Lowenthal MN. Chronic myelocytic leukemia. Origin of some lymphocytes from leukemic stem cells. J Clin Invest 1978;62:815-23.
7. Bedi A, Zehnbauer BA, Collector MI, Barber JP, Zicha MS, Sharkis SJ, Jones RJ. BCR-ABL gene rearrangement and expression of primi-

tive hematopoietic progenitors in chronic myeloid leukemia. Blood 1993;81:2898-902.
8. Raaijmakers MH, van Emst L, de Witte T, Mensink E, Raymakers RA. Quantitative assessment of gene expression in highly purified hematopoietic cells using real-time reverse transcriptase polymerase chain reaction. Exp Hematol 2002;30:481-7.
9. Mahon FX, Belloc F, Lagarde V, Chollet C, Moreau-Gaudry F, Reiffers J, Goldman JM, Melo JV. MDR1 gene overexpression confers resistance to imatinib mesylate in leukemia cell line models. Blood 2003;101:2368-73.
10. Keating A, Wang XH, Laraya P. Variable transcription of BCR-ABL by Ph+ cells arising from hematopoietic progenitors in chronic myeloid leukemia. Blood 1994;83:1744-9.
11. Holyoake T, Jiang X, Eaves C, Eaves A. Isolation of a highly quiescent subpopulation of primitive leukemic cells in chronic myeloid leukemia. Blood 1999;94:2056-64.
12. Jiang G, Yang F, Li M, Weissbecker K, Price S, Kim KC, La Russa VF, Safah H, Ehrlich M. Imatinib (ST1571) provides only limited selectivity for CML cells and treatment might be complicated by silent BCR-ABL genes. Cancer Biol Ther 2003;2:103-8.
13. Mahon FX, Delbrel X, Cony-Makhoul P, Faberes C, Boiron JM, Barthe C, Bilhou-Nabera C, Pigneux A, Marit G, Reiffers J. Follow-up of complete cytogenetic remission in patients with chronic myeloid leukemia after cessation of interferon alfa. J Clin Oncol 2002;20:214-20.
14. Kantarjian HM, O'Brien S, Cortes JE, Shan J, Giles FJ, Rios MB, Faderl SH, Wierda WG, Ferrajoli A, Verstovsek S, Keating MJ, Freireich EJ, Talpaz M. Complete cytogenetic and molecular responses to interferon-alpha-based therapy for chronic myelogenous leukemia are associated with excellent long-term prognosis. Cancer 2003;97:1033-41.
15. Kujawski LA, Talpaz M. The role of interferon-alpha in the treatment of chronic myeloid leukemia. Cytokine Growth Factor Rev 2007;18:459-71.
16. Pierce A, Smith DL, Jakobsen LV, Whetton AD, Spooncer E. The specific enhancement of interferon alpha induced growth inhibition by BCR/ABL only occurs in multipotent cells. Hematol J 2001;2:257-64.
17. Angstreich GR, Matsui W, Huff CA, Vala MS, Barber J, Hawkins AL, Griffin CA, Smith BD, Jones RJ. Effects of imatinib and interferon on primitive chronic myeloid leukaemia progenitors. Br J Haematol 2005;130:373-81.
18. Guilhot F, Roy L, Saulnier PJ, Guilhot J. Interferon in chronic myeloid leukaemia: past and future. Best Pract Res Clin Haematol 2009;22:315-29.
19. Burchert A, Neubauer A. Interferon alpha and T-cell responses in chronic myeloid leukemia. Leuk Lymphoma 2005;46:167-75.
20. Burchert A, Wolfl S, Schmidt M, Brendel C, Denecke B, Cai D, Odyvanova L, Lahaye T, Muller MC, Berg T, Gschaidmeier H, Wittig B, Hehlmann R, Hochhaus A, Neubauer A. Interferon-alpha, but not the ABL-kinase inhibitor imatinib (STI571), induces expression of myeloblastin and a specific T-cell response in chronic myeloid leukemia. Blood 2003;101:259-64.
21. Molldrem JJ, Lee PP, Wang C, Felio K, Kantarjian HM, Champlin RE, Davis MM. Evidence that specific T lymphocytes may participate in the elimination of chronic myelogenous leukemia. Nat Med 2000;6:1018-23.
22. Gannage M, Abel M, Michallet AS, Delluc S, Lambert M, Giraudier S, Kratzer R, Niedermann G, Saveanu L, Guilhot F, Camoin L, Varet B, Buzyn A, Caillat-Zucman S. Ex vivo characterization of multiepitopic tumor-specific CD8 T cells in patients with chronic myeloid leukemia: implications for vaccine development and adoptive cellular immunotherapy. J Immunol 2005;174:8210-8.
23. van Dongen JJ, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, Gottardi E, Rambaldi A, Dotti G, Griesinger F, Parreira A, Gameiro P, González Diáz M, Malec M, Langerak AW, San Miguel JF, Biondi A. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 1999;13:1901-28.
24. Faber E, Naušová J, Jarošová M, Egorin MJ, Holzerová M, Rožmanová Š, Marešová I, Divoký V, Indrák K. Intermittent dosage of imatinib mesylate in CML patients with a history of significant hematologic toxicity after standard dosing. Leuk Lymphoma 2006;47:1082-90.
25. Cortes JE, Talpaz M, O'Brien S, Faderl S, Garcia-Manero G, Ferrajoli A, Verstovsek S, Rios MB, Shan J, Kantarjian HM. Staging of chronic myeloid leukemia in the imatinib era: an evaluation of the World Health Organization proposal. Cancer 2006;106:1306-15.
26. Sokal JE, Cox EB, Baccarani M, Tura S, Gomez GA, Robertson JE, Tso CY, Braun TJ, Clarkson BD, Cervantes F, Et A. Prognostic discrimination in "good-risk" chronic granulocytic leukemia. Blood 1984;63:789-99.
27. Hasford J, Pfirrmann M, Hehlmann R, Allan NC, Baccarani M, Kluin-Nelemans JC, Alimena G, Steegmann JL, Ansari H. A new prognostic score for survival of patients with chronic myeloid leukemia treated with interferon alfa. Writing Committee for the Collaborative CML Prognostic Factors Project Group. J Natl Cancer Inst 1998;90:850-8.
28. Baccarani M, Cortes J, Pane F, Niederwieser D, Saglio G, Apperley J, Cervantes F, Deininger M, Gratwohl A, Guilhot F, Hochhaus A, Horowitz M, Hughes T, Kantarjian H, Larson R, Radich J, Simonsson B, Silver RT, Goldman J, Hehlmann R. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. J Clin Oncol 2009;27:6041-51.
29. Cox DR. Regression models and life tables (with discussion). J Royal Statist Soc Ser B 1972;34:187-220.
30. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.
31. Talpaz M, Kantarjian H, Kurzrock R, Trujillo JM, Gutterman JU. Interferon-alpha produces sustained cytogenetic responses in chronic myelogenous leukemia. Philadelphia chromosome-positive patients. Ann Intern Med 1991;114:532-8.
32. Hehlmann R. Introduction: CML in the imatinib era. Best Pract Res Clin Haematol 2009;22:283-4.
33. Faber E, Kuba A, Zapletalová J, Divoká M, Rožmanová Š, Rohoň P, Holzerová M, Jarošová M, Indrák K on behalf of cooperating group. Operational cures after interferon-alpha in patients with chronic myeloid leukemia in Central and Northern Moravia. J Interferon Cytokine Res 2012;32:230-4.
34. Cortes J, Quintas-Cardama A, Jones D, Ravandi F, Garcia-Manero G, Verstovsek S, Koller C, Hiteshew J, Shan J, O'Brien S, Kantarjian H. Immune modulation of minimal residual disease in early chronic phase chronic myelogenous leukemia: a randomized trial of frontline high-dose imatinib mesylate with or without pegylated interferon alpha-2b and granulocyte-macrophage colony-stimulating factor. Cancer 2011;117:572-80.
35. Nicolini FE, Hayette S, Legros L, Rousselot P, Maloisel F, Tulliez M, Guerci A, Charbonnier A, Prebet T, Rigal-Huguet F, Chabane K, Magaud JP, Paillet C, Pivot C, Michallet M. Pegylated IFN-alpha2a combined to imatinib mesylate 600mg daily can induce complete cytogenetic and molecular responses in a subset of chronic phase CML patients refractory to IFN alone or to imatinib 600mg daily alone. Leuk Res 2011;35:80-6.
36. Preudhomme C, Guilhot J, Nicolini FE, Guerci-Bresler A, Rigal-Huguet F, Maloisel F, Coiteux V, Gardembas M, Berthou C, Vekhoff A, Rea D, Jourdan E, Allard C, Delmer A, Rousselot P, Legros L, Berger M, Corm S, Etienne G, Roche-Lestienne C, Eclache V, Mahon F-X, Guilhot F for the SPIRIT Investigators and France Intergroupe des Leucémies Myéloïdes Chroniques (Fi-LMC). Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. N Engl J Med 2010;363:2511-21.
37. Burchert A, Muller MC, Kostrewa P, Erben P, Bostel T, Liebler S, Hehlmann R, Neubauer A, Hochhaus A. Sustained molecular response with interferon alfa maintenance after induction therapy with imatinib plus interferon alfa in patients with chronic myeloid leukemia. J Clin Oncol 2010;28:1429-35.
38. Mahon FX, Rea D, Guilhot J, Guilhot F, Huguet F, Nicolini F, Legros L, Charbonnier A, Guerci A, Varet B, Etienne G, Reiffers J, Rousselot P. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol 2010;11:1029-35.
39. Kreutzman A, Rohon P, Faber E, Indrak K, Juvonen V, Kairisto V, Voglova J, Sinisalo M, Flochova E, Vakkila J, Arstila P, Porkka K, Mustjoki S. Chronic myeloid leukemia patients in prolonged remission following interferon-a monotherapy have distinct cytokine and oligoclonal lymphocyte profile. PLoS ONE 2011;6:e23022.