Praćenje odgovora bolesnika oboljelih od kronične mijeloične leukemije na liječenje inhibitorima tirozin-kinaze kvantitativnom lančanom reakcijom polimeraze u stvarnom vremenu

Monitoring chronic myeloid leukemia patients responding to treatment with tyrosine kinase inhibitors by real-time quantitative polymerase chain reaction

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Sažetak

Uvod: Kroz povijest je terapija kronične mijeloične leukemije (engl. *chronic myeloid leukemia*, CML) imala puno revolucionarnih pomaka, među kojima je najvažniji uvođenje imatinib mesilata (engl. *imatinib mesylate*, IM). Preciznija procjena terapijskog odgovora na IM i točno mjerenje stupnja smanjenja prijepisa BCR-ABL, može se postići upotrebom kvantitativne lančane reakcije polimeraze u stvarnom vremenu (engl. *real-time quantitative polymerase chain reaction*, RQ-PCR).

Cilj: Kvantificirati prijepise BCR-ABL kod bolesnika s CML i promatrati odgovor na liječenje inhibitorima tirozin-kinaze.

Materijali i metode: U istraživanje je bio uključen 31 bolesnik liječen pomoću IM. Napravljena je RQ-PCR prema protokolu *Europe Against Cancer* s ABL kao „kućepaziteljskim" genom (engl. *housekeeping gene*) i izračunat je omjer BCR-ABL/ABL. Bolesnici su podijeljeni u skupine prema kriterijima Europske mreže za leukemiju (engl. *European Leukemia Net*) za postizanje značajnog molekularnog odgovora (engl. *major molecular response*, MMoR). I. skupina sastojala se od 11 bolesnika sa smanjenjem višim od 3 log, II. skupina od 13 bolesnika sa smanjenjem nižim od 3 log, a III. skupina od 7 bolesnika koji su bili manje od 18 mjeseci na kontrolama i praćenju.

Rezultati: Bolesnici I. skupine, koji su bili pozitivni ili negativni na prijepis BCR-ABL, odgovarali su na terapiju u vremenu od 2 godine praćenja i kontrola te su ispunili kriterije za povoljnu dugoročnu prognozu. Bolesnici II. skupine nisu odgovarali na terapiju pomoću IM te su zahtijevali drugačiji terapijski pristup, višu dozu istog inhibitora tirozin-kinaze ili drugu generaciju lijeka. Sedmoro bolesnika III. skupine kojima je nedavno dijagnosticirana bolest, praćeni su manje od 18 mjeseci pa im se MMoR nije mogao procijeniti. Primjenom iste analogije kao kod prve dvije skupine, može se napraviti prognoza tijeka bolesti.

Zaključak: Naši rezultati pokazuju da je RQ-PCR obvezna metoda za pažljivo promatranje odgovora na liječenje inhibitorima tirozin-kinaze, radi omogućavanja ispravne terapije te kako bi se odlučilo treba li mijenjati terapiju, a u slučaju da treba, kada to učiniti.

Ključne riječi: kronična mijeloična leukemija; imatinib mesilat; kvantitativna lančana reakcija polimeraze; značajni molekularni odgovor

Abstract

Introduction: Historically, many revolutionary advances in therapy for chronic myeloid leukemia (CML) have been achieved over time, among them most important being imatinib mesylate (IM). More precise assessment of response to therapy with IM and an accurate measure of the degree of BCR-ABL transcript reduction can be achieved by using real-time quantitative polymerase chain reaction (RQ-PCR).

Aim: To quantitate BCR-ABL transcripts in CML patients and to monitor response to treatment with tyrosine kinase inhibitors.

Materials and methods: The study included a 31 patients treated with IM. RQ-PCR was performed according to the Europe Against Cancer protocol, with ABL as a housekeeping gene. BCR-ABL/ABL ratio was calculated. Patients were divided into groups according to the European Leukemia Net criteria for achievement of major molecular response (MMoR). Group I consisted of 11 patients with more than 3 log reduction, group II consisted of 13 patients with less than 3 log reduction, and group III included 7 patients with a follow up of less than 18 months.

Results: Group I patients achieved MMoR with detectable or undetectable BCR-ABL transcript in a period of 2 years of follow up and fulfilled the criteria for favorable long term prognosis. Group II patients never achieved MMoR with IM and required different therapy approach, higher dose of the same tyrosine kinase inhibitor or a second generation drug. Seven newly diagnosed CML patients from group III were monitored for less than 18 months and therefore MMoR could not be estimated. Using the same analogy as in the first two groups, prediction of the course of disease could be possible.

Conclusion: Study results show that RQ-PCR is mandatory for careful monitoring of therapeutic response to tyrosine kinase inhibitors in order to ensure that an individual patient receives proper treatment and to decide whether and when therapy should be changed.

Key words: chronic myeloid leukemia; imatinib mesylate; quantitative polymerase chain reaction; major molecular response

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Uvod

Kronična mijeloična leukemija (engl. *chronic myeloid leukemia*, CML) otkrivena je u prvoj polovini 19. stoljeća (1). Ovaj tip leukemije obilježava pojačana granulocitopoeza u koštanoj srži i perifernoj krvi. Bitan znak CML je tzv. filadelfijski kromosom (engl. *Philadelphia chromosome*, *Ph chromosome*), rezultat translokacije t(9;22) najvjerojatnije u jednoj hematopoetskoj matičnoj stanici. Taj tip preraspodjele gena s uskog dijela kromosoma (engl. *breakpoint cluster region,* BCR) na kromosomu 22 na područje kinaze ABL na kromosomu 9, rezultira stvaranjem fuzijskog gena *BCR-ABL*. Proizvod fuzijskog gena je protein BCR-ABL, čija prejaka tirozin-kinazna aktivnost stimulira povećano stvaranje stanica (2).

Kroz povijest je bilo puno revolucionarnih pomaka u terapiji CML. Jedan od najčešće upotrebljavanih lijekova 50-ih godina prošloga stoljeća bio je busulfan, no nakratko je zamijenjen hidroksikarbamidom. Za interferon-alfa, koji je uveden u terapiju 80-tih godina, dokazano je da dovodi do negativnog nalaza Ph kromosoma u koštanoj srži. Između 1980. i 2000. presađivanje koštane srži bila je prva terapijska opcija kod mladih bolesnika s HLA-podudarnim darivateljima (engl. *HLA-matched donors*). Najvažnije otkriće u liječenju bio je inhibitor tirozin-kinaze ABL, imatinib mesilat (engl. *imatinib mesylate*, IM). IM je 2-fenilaminopirimidinski spoj, mala molekula koja uspješno blokira kinaznu aktivnost proteina BCR-ABL. IM zauzima mjesto vezanja ATP u proteinu BCR-ABL, uzrokujući zatvorenu konfiguraciju i inhibiciju enzimske aktivnosti proteina (2). IM se uzima oralno s početnom dozom od 400 mg na dan i zlatni je standard za prvu terapiju kod bolesnika s tek dijagnosticiranom CML, zbog ograničene toksičnosti i visoke stope povoljnog terapijskog odgovora.

Učestalost potpunog citogenetičkog odgovora (engl. *complete cytogenetic response*, CCyR) vrlo je visoka kod bolesnika koji su uzimali IM, što se vidi iz činjenice da u praćenim uzorcima nije nađena pozitivna Ph metafaza. Jednom kada bolesnik postigne CCyR, kvantitativna lančana reakcija polimerazom u stvarnom vremenu (engl. *real-time quantitative polymerase chain reaction*, RQ-PCR) je metoda koja daje najviše informacija o količini prijepisa BCR-ABL (1,4). Kod ove je metode broj prijepisa BCR-ABL u korelaciji s brojem prijepisa kontrolnog gena (ABL), koji je u stanicama zahvaćenim leukemijom izražen u približno istoj količini kao i u zdravim stanicama. Općenito se rezultat izražava kao logaritam smanjenja omjera BCR-ABL/ABL. Smanjenje od 3 ili više log omjera BCR-ABL/ABL unutar 18 mjeseci zove se značajnim molekularnim odgovorom (engl. *major molecular response,* MMoR) (2,4). Pokazalo se da je točno mjerenje smanjenja prijepisa BCR-ABL obrnuto proporcionalno s vjerojatnošću progresije bolesti (1).

Introduction

Chronic myeloid leukemia (CML) was discovered in the first half of the 19th century (1). This type of leukemia is characterized by abnormal granulopoiesis in bone marrow and peripheral blood. Important landmark of CML is Philadelphia chromosome (Ph chromosome), a result of a t(9;22) translocation, probably in a single hematopoietic stem cell. This type of gene rearrangement of breakpoint cluster region (BCR) on chromosome 22 to ABL kinase domain on chromosome 9 leads to development of a *BCR-ABL* fusion gene. The product of this fusion gene, BCR-ABL protein, has abnormal tyrosine kinase activity stimulating enlarged cell proliferation (2).

Historically, many revolutionary advances in therapy for chronic myeloid leukemia (CML) have been achieved over time. One of the most frequently used drugs for CML in the 1950s was busulfan, but it was shortly replaced with hydroxycarbamide. Interferon-alpha, introduced in therapy in the 1980s, was the first agent to induce any degree of Ph-chromosome negativity in bone marrow. Between 1980 and 2000, bone marrow transplantation was the first therapeutic option in young patients with HLA-matched donors. The most important therapeutic discovery was original ABL tyrosine kinase inhibitor, imatinib mesylate (IM). IM is a 2-phenylaminopyrimidine compound, a small molecule that effectively blocks the kinase activity of BCR-ABL protein. IM occupies the ATP-binding site of BCR-ABL protein, causing a closed configuration and inhibition of the enzyme activity of the protein (2). IM is administered orally with the initial dose of 400 mg *per* day and is the gold standard as first line therapy in newly diagnosed CML patients, because of its limited toxicity and high response rate (3).

The frequency of complete cytogenetic response (CCyR) is very high in IM-treated patients, which is evident from the fact that no Ph positive metaphase is found in the follow up sample. Once the patient has achieved CCyR, the most informative method to measure the level of BCR-ABL transcripts is real-time quantitative polymerase chain reaction (RQ-PCR) (1,4). In this methodology, the BCR-ABL transcript numbers are related to transcript numbers of a control gene (ABL) that is expressed in leukemia cells at approximately the same level as in normal cells. The result is generally expressed as log reduction in the BCR-ABL/ABL ratio, and achievement of 3 or more log reduction in BCR-ABL/ABL ratio in 18 months is called major molecular response (MMoR) (2,4). It has been shown that accurate measurement of BCR-ABL transcript reduction correlates inversely with the probability of disease progression (1). Furthermore, patients in MMoR can remain positive and stable, or can have undetectable BCR-ABL transcript during follow up. For non-responders, or those who never achieve MMoR, monitoring of the BCR-ABL/ABL ratio is cru-

Nadalje, bolesnici koji su odgovorili na terapiju mogu ostati pozitivni i stabilni ili negativni za prijepis BCR-ABL tijekom kontrola i praćenja. Za bolesnike koji ne odgovaraju na lijek ili za one koji nikada ne odreagiraju na terapiju, praćenje omjera BCR-ABL/ABL od velike je važnosti, kako bi se mogla revidirati strategija terapije. Velik dio tih bolesnika razvija otpornost na lijek, što je uzrokovano mutacijama na području kinaze ABL (5) ili pokazuju nedefinirane individualne farmakokinetičke varijacije. Moguće liječenje takvih bolesnika jest povećanje doze IM (600 ili 800 mg na dan), korištenje inhibitora tirozin-kinaze druge generacije (nilotinib, dasatinib) ili presađivanje koštane srži (2).

Cilj ovoga istraživanja bio je procijeniti djelotvornost protokola liječenja i postići bolju procjenu odgovora pojedinih bolesnika na liječenje pomoću IM primjenom osjetljive tehnike mjerenja omjera BCR-ABL/ABL metodom kvantitativne lančane reakcije polimeraze, kako bi se bolesnici stratificirali prema riziku od relapsa bolesti te kako bi se individualizirao protokol liječenja.

Materijali i metode

U istraživanje je bio uključen 31 bolesnik (13 žena i 18 muškaraca) s dijagnozom CML. Desetoro bolesnika bilo je prethodno liječeno busulfanom ili interferonom-alfa, dok je kod 21 bolesnika CML bila tek nedavno dijagnosticirana. Postavljanje dijagnoze i liječenje odvijalo se na Klinici za unutarnje bolesti Kliničkog bolničkog centra Zagreb. Početna doza IM za sve bolesnike bila je 400 mg na dan. Bolesnici su praćeni 39 mjeseci (medijan; raspon 3–81).

RNA je izolirana iz koštane srži ili stanica periferne krvi pomoću komercijalnog reagens paketa QuickPrep mRNA purification kit (GE Healthcare, UK) prema uputama proizvođača. RQ-PCR je provedena prema protokolu EAC uz primjenu tehnologije TaqMan (LightCycler, Roche) (6). Reverzna transkripcija RNA odvijala se u volumenu reakcijske smjese od 20 µL uporabom FirstStrand cDNA synthesis kit (Amersham Biosciences, UK). Amplifikacija fuzijskog gena BCR-ABL i ABL kao kontrolnog gena (RQ-PCR) napravljena je setom Fusion*Quant* Kit za analizu gena BCR-ABL metodom RQ-PCR Mbcr (Ipsogen, France) prema uputama proizvođača. Svi su uzorci fuzijskog gena BCR-ABL i gena ABL analizirani tri puta te je izračunat omjer BCR-ABL/ABL (7).

Od neliječenih bolesnika pri dijagnozi je iz odnosa BCR-ABL/ABL dobivena standardizirana osnovna vrijednost koja je zatim upotrebljena za izračun log vrijednosti pada fuzijskog prijepisa u svim uzorcima u vremenu praćenja (1,6)

Bolesnici su podijeljeni u skupine prema izračunatom log smanjenju omjera BCR-ABL/ABL tijekom 18 mjeseci promatranja na IM terapiji. U I. skupini bilo je 11 bolesnika koji su postigli više od 3 log smanjenja u 18 mjeseci, u II. skupini 13 bolesnika koji su imali manje od 3 log smanjenja u 18 mjeseci IM terapije, a u III. skupini 7 bolesnika koji su praćeni manje od 18 mjeseci.

cial to revise therapeutic strategy. A high proportion of these patients develop resistance to the drug caused by ABL kinase domain mutations (5), or show undefined individual variations in pharmacokinetics. In these patients, optional treatment is increment in IM dose (600 or 800 mg/day), second generation tyrosine kinase inhibitors (nilotinib, dasatinib), or bone marrow transplantation (2).

The aim of this study was to evaluate the efficacy of a treatment protocol and to achieve better assessment of the individual patient response to treatment with IM by using sensitive quantitative PCR measurement of the BCR-ABL/ABL ratio, in order to stratify patients according to the risk of relapse, and to individualize treatment protocol.

Materials and methods

The study included 31 patients (13 women and 18 men) diagnosed with CML. Ten of these patients were pretreated with busulfan or interferon-alpha, and the remaining 21 patients were newly diagnosed CML patients. Patients were diagnosed and treated at Hematology Department, University Department of Medicine, Zagreb University Hospital Center. Initial IM dose for all patients was 400 mg/day. Patients were followed up for 39 (3–81) months; median (range).

RNA was isolated from bone marrow or peripheral blood cells by using QuickPrep mRNA purification kit (GE Healthcare, UK) according to the manufacturer's instructions.

RQ-PCR was performed according to EAC protocol using TaqMan technology (LightCycler, Roche) (6). RNA was reverse transcribed in reaction volume of 20 µL using FirstStrand cDNA synthesis kit (Amersham Biosciences, UK). Amplification of fusion BCR-ABL and ABL as a control gene (RQ-PCR) was performed using Fusion*Quant* Kit for RQ-PCR analysis of BCR-ABL Mbcr (Ipsogen, France) according to the manufacturer's instructions. All samples were analyzed in triplicates for both BCR-ABL and ABL, and the BCR-ABL/ABL ratio was calculated (7).

Standardized baseline was derived from the BCR-ABL/ABL ratio obtained from untreated patients at diagnosis and was used for calculation of log reduction for all follow up samples (1,6).

Patients were divided into groups according to calculated log reduction of the BCR-ABL/ABL ratio during 18-month follow up of IM therapy. Group I consisted of 11 patients that achieved more than 3 log reduction in 18 months; group II included 13 patients that had less than 3 log reduction in 18 months of IM therapy; and group III included 7 patients with a follow up of less than 18 months.

pini bilo je 13 bolesnika koji su imali smanjenje manje od 3 log u 18 mjeseci terapije pomoću IM, dok je u III. skupini bilo 7 bolesnika koji su bili na promatranju manje od 18 mjeseci.

Rezultati

Ukupno je 31 bolesnik s dijagnosticiranom CML započeo terapiju pomoću IM (400 mg na dan) i bio na promatranju 39 mjeseci (medijan; raspon 3–81). Tijekom razdoblja promatranja i odlazaka na kontrole 6 od 31 bolesnika bilo je negativno za prijepis BCR-ABL, što je izmjereno pomoću RQ-PCR. Rezultati stope terapijskog odgovora na IM unutar 18 mjeseci za I. i II. skupinu te posljednji uzorak promatranja III. skupine prikazani su na slici 1.

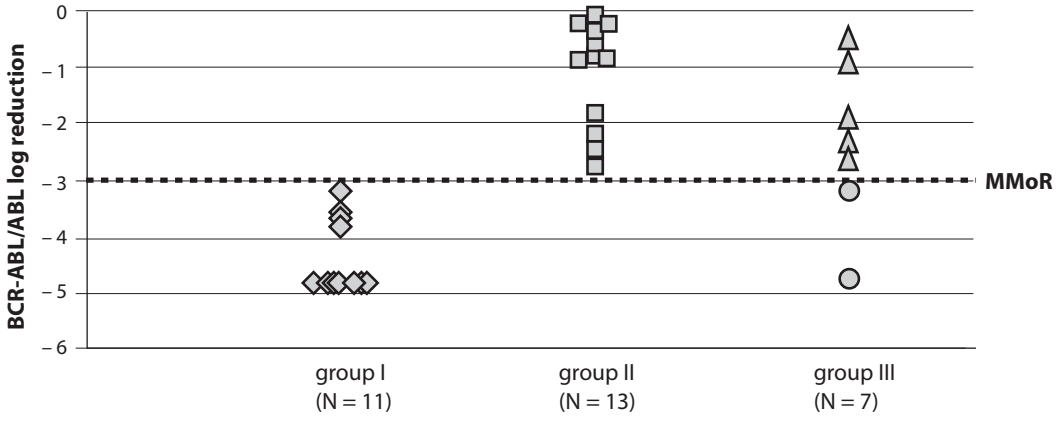
Rezultati 11 bolesnika (I. skupina) koji su postigli smanjenje od 3 ili više log i time ispunili kriterije za MMoR prikazani su u tablici 1. Shematski prikaz promatranja IM terapije i reakcije na terapiju u toj skupini nalazi se na slici 2a. Ti su bolesnici bili promatrani tijekom 39 mjeseci (medijan; raspon 22–63) a MMoR su zadržali 21 mjesec (medijan; raspon 4–45). Od trenutka postizanja MMoR kod šestoro tih bolesnika, uključujući i troje prethodno liječenih busulfanom i interferonom-alfa, prijepis BCR-ABL je bio negativan tijekom 25 mjeseci (medijan; raspon 4–45). Niti kod jednog od tih bolesnika nije bilo recidiva bolesti. Ostalih četvoro bolesnika bilo je periodično pozitivno na prijepis BCR-ABL u promatranom vremenu od 29 mjeseci (medijan; raspon 11–42). Jedan od njih više nije reagirao na terapiju te je, nakon 11 mjeseci promatranja i kontrola, došlo do recidiva. Samo je jedan bolesnik pozitivan za prijepis BCR-ABL odgovorio na terapiju tijekom 14 mjeseci, u jednom trenutku odgovor se izgubio, no nije došlo do recidiva.

Results

A total of 31 patients diagnosed with CML that started using therapy with IM (400 mg/day) were followed-up for a median time of 39 (range 3–81) months. During the follow up period, six of 31 patients reached an undetectable BCR-ABL transcript by RQ-PCR. Results on the response rate to IM therapy at 18 months for groups I and II and in the last follow up sample for group III are presented in Figure 1.

Results on 11 patients (group I) that achieved 3 or more log reduction and met the criteria for MMoR are presented in Table 1. Schematic presentation of IM therapy monitoring and MMoR achievement in this group is shown in Figure 2a. These patients were monitored for a median time of 39 (range 22–63) months, and remained in MMoR for 21 (range 4–45) months. Six of these patients, including three patients pretreated with busulfan and interferon-alpha, having achieved MMoR, maintained undetectable BCR-ABL transcript for a median time of 25 (range 4–45) months. None of these patients relapsed. Another four patients had time points with detectable BCR-ABL transcript during the monitoring period (median time) of 29 (range 11–42) months. One of them lost MMoR and relapsed after 11-month follow up. Only one patient achieved MMoR with detectable BCR-ABL transcript during 14 months and at one time point lost response but never relapsed.

Results on 13 patients (group II) that did not achieve MMoR in 18 months of IM therapy are presented in Table 2. Schematic presentation of IM therapy monitoring without achievement of MMoR in group II is shown in Figure 2b. In these patients, therapeutic modification occurred after 27 months of follow up (range 7–67): 6 patients star-



Slika 1. Log smanjenje omjera BCR-ABL/ABL u ispitivanim grupama dosegnuto u 18 mjeseci (grupa I i II) i u uzorcima praćenim <18 mjeseci (grupa III). MMoR – glavni molekularni odgovor.

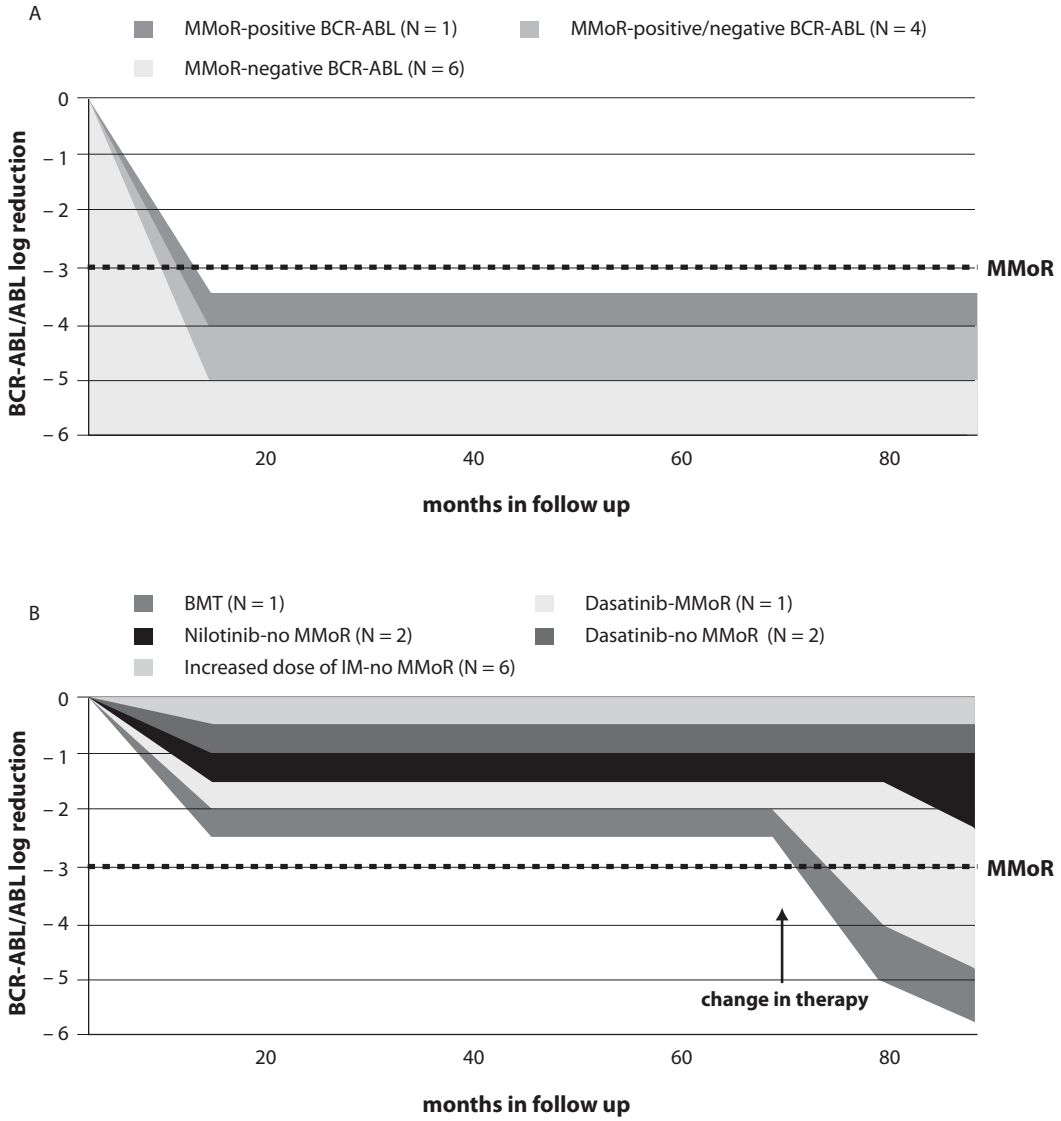
Figure 1. BCR-ABL/ABL log reduction in study groups achieved at 18 months (groups I and II) and in the follow up sample (group III). MMoR – major molecular response

Tablica 1. Rezultati praćenja grupe I.

Table 1. Follow up results in group I

>3 log reduction in 18 months N = 11	Follow-up (months)	No. of patients with loss of MMoR	No. of patients in relapse
Follow up	39 (22–63)		
MMoR	21 (4–45)	3	1
MMoR (BCR-ABL negative) N = 6	25 (4–45)	1	0
MMoR (BCR-ABL positive/negative) N = 4	29 (11–42)	1	1
MMoR (BCR-ABL positive) N = 1	14	1	0

MMoR - major molecular response



Slika 2. Praćenje bolesnika na terapiji s IM: a) odgovor na terapiju IM u grupi I, b) bez odgovora na terapiju IM u grupi II. BMT – transplantacija koštane srži, MMoR – glavni molekularni odgovor

Figure 2. Monitoring patients on IM therapy: (a) achievement of major molecular response (MMoR) in group I; (b) MMoR was never achieved in group II. BMT – bone marrow transplantation; MMoR – major molecular response

Tablica 2. Rezultati praćenja grupe II.

Table 2. Follow up results in group II.

<3 log reduction in 18 months (N = 13)		Follow-up (months)	Log reduction	No. of patients with MMoR
Follow up		27 (7–67)		
Change in therapy	Increased dose of IM (N = 6)*	15 (6–41)	1.7 (0.7–2.5)	0
	Nilotinib (N = 2)	5 and 10	2.2 and 0.2	0
	Dasatinib (N = 3)*	14 (9–25)	0.8–4.8	1
	BMT (N = 1)	11	4.8	1
	Unknown (N = 2)	NA	NA	NA

*one of the patients was in both IM increased dose therapy group and in dasatinib group; BMT – bone marrow transplantation; MMoR – major molecular response; NA – not available

Rezultati 13 bolesnika (II. skupina) koji u 18 mjeseci nisu odgovorili na IM terapiju prikazani su u tablici 2. Shematski prikaz promatranja IM terapije bez postignutog MMoR u II. skupini nalazi se na slici 2b. Kod ovih bolesnika promjene u terapiji dogodile su se nakon 27-mjesečnog promatranja (raspon 7–67 mjeseci) kad je šest bolesnika započelo s povećanom dozom IM (600 ili 800 mg na dan). U razdoblju praćenja od 15 mjeseci (raspon 6–41) nitko od njih nije pozitivno odgovorio na terapiju. Jedan od ovih bolesnika započeo je uzimati dasatinib, nakon što se pokazalo da IM terapija nije uspješna (vrijeme praćenja 41 mjesec) i u 9 mjeseci je postigao više od 3 log smanjenja. Ostala dva bolesnika na terapiji dasatinibom postigla su manje od jednog log smanjenja u 14, odnosno 25 mjeseci praćenja i dolazaka na kontrolu. Druga su dva bolesnika bila na terapiji nilotinibom, od kojih je jedan u 10 mjeseci postigao smanjenje od samo 0,2 log, a drugi 2,2 log u 5 mjeseci. Bolesnik negativan za prijepis BCR-ABL, kod kojega nije došlo do odgovora na IM terapiju, bio je 11 mjeseci kasnije podvrgnut presađivanju koštane srži. Sedmoro bolesnika iz ove skupine bilo je prethodno liječeno busulfanom i interferonom-alfa. Petoro od njih, kao i dvoje od šestoro bolesnika sa novodijagnosticiranom CML, nisu odgovorili na promjenu terapije. Za dva bolesnika koji su prethodno liječeni busulfanom i interferonom-alfa ne postoje podaci o tijeku terapije.

III. skupina uključivala je sedam bolesnika kojima je nedavno dijagnosticirana CML (Tablica 3.) i koji su bili na promatranju manje od 18 mjeseci (medijan 8 mjeseci, raspon 3–12 mjeseci) te im se stoga MMoR nije mogao procijeniti. Dvoje od njih postiglo je smanjenje 3 log u 3, odnosno 10 mjeseci praćenja, ostalo dvoje postiglo je smanjenje od 2 log u 3, odnosno 8 mjeseci. Posljednja 3 bolesnika postigla su smanjenje manje od 2 log u 6 mjeseci (medijan, raspon 3–12).

ted with an increased IM dose (600 or 800 mg/day). In the monitoring period of 15 months (range 6–41), none of them achieved MMoR. One of these patients started dasatinib after failure of increased IM therapy dose (monitoring time 41 months) and in 9 months achieved more than 3 log reduction. Another two patients on dasatinib therapy achieved less than one log reduction in 14 and 25 months of follow up. Two other patients started nilotinib therapy; one achieved only 0.2 log reduction in 10 months and the other 2.2 log reduction in 5 months. The patient without any response to IM therapy underwent bone marrow transplantation with undetectable BCR-ABL transcript 11 months later. Seven patients from this group were pretreated with busulfan and interferon-alpha. Five of them as well as two of the six newly diagnosed CML patients did not respond to therapy modification. For two pretreated patients in this group, there are no data on the course of therapy.

Group III included seven newly diagnosed CML patients (Table 3) monitored for less than 18 months (8 months, range 3–12 months) and therefore MMoR could not be estimated. Two of them achieved 3 log reduction at 6 and 10 months of follow up, the other two achieved 2 log reduction in 3 and 8 months. The last three patients achieved less than 2 log reduction in the median time of 6 (range 3–12) months.

Discussion

Measurement is fundamental in hematology, especially in case of leukemia where counting and identifying cells in blood and bone marrow are traditional (8). For many years, response to CML therapy was measured with a number of leukemic cells originating from abnormal granulopoiesis. With time, improvement in therapy required ever more sensitive techniques for better assessment of

Tablica 3. Rezultati praćenja grupe III.

Table 3. Follow up results in group III.

<18 month follow-up (N = 7)	Follow-up (months)	Log reduction
Follow up	8 (3–12)	0.5–4.8
>3 log reduction (N = 2)	6 and 10	3.1 and 4.8
>2 log reduction (N = 2)	3 and 8	2.3 and 2.6
<2 log reduction (N = 3)	6 (3–12)	0.5–1.9

Rasprava

U hematologiji su metode mjerenja osnovni alati, naročito u slučaju leukemije, gdje su brojanje i identifikacija stanica u krvi i koštanoj srži tradicionalne metode (8). Dugo se odgovor na terapiju za CML mjerio brojem stanica zahvaćenih leukemijom koje potiču od pojačane granulocitopoeze. S vremenom je poboljšanje terapije počelo zahtijevati osjetljivije tehnike za bolju procjenu odgovora (9). Šezdesetih godina prošloga stoljeća otkrivena je specifična anomalija kromosoma u CML, poznata kao Ph kromosom. Identifikacija i kvantifikacija Ph pozitivnih metafaza postala je dragocjen podatak pri potvrđivanju dijagnoze i praćenju terapijskog odgovora (1,2). Međutim, želja za daljnjim unapređenjem osjetljivosti dovela je do uvođenja molekularnih tehnika za mjerenje prijepisa BCR-ABL. Otkriće inhibitora tirozin-kinaze ABL, imatinib mesilata, bila je revolucionaran korak u liječenju CML a jedina metoda za mjerenje razine postignute remisije je kvantitativna lančana reakcija polimerazom u stvarnom vremenu (2,7,11). Mjerenje razine prijepisa BCR-ABL, izraženog kao log smanjenja omjera BCR-ABL/ABL, rabi se u svrhu omogućavanja bolje procjene terapijskog odgovora na IM pojedinačnih bolesnika te za ocjenu djelotvornosti protokola liječenja. Može se rabiti i za stratifikaciju bolesnika prema riziku od pojave recidiva, a presudan je u preispitivanju terapijske strategije.

Iako potpuna procjena odgovora prema udruženju Europske mreže za leukemiju (engl. *European Leukemia Net*) uključuje kliničku procjenu, krvnu sliku, citogenetičku analizu koštane srži i izračunavanje omjera BCR-ABL/ABL svaka 3 mjeseca od početka terapije (2), u ovom istraživanju je za promatranje bolesnika s CML u standardnom protokolu liječenja pomoću IM primijenjeno samo log smanjenje omjera BCR-ABL/ABL. Općenito, smanjenje omjera BCR-ABL/ABL u bliskoj je uzajamnoj vezi s zadovoljavajućim hematološkim i citogenetičkim odgovorom. Poznato je da je smanjenje omjera BCR-ABL/ABL od 2 log tijekom 12 mjeseci promatranja u skladu s Ph negativnim nalazom u koštanoj srži (CCyR) (10). U tom je razdoblju RQ-PCR jedini

therapeutic response (9). In the 1960s, specific CML chromosome abnormality, known as Ph chromosome, was discovered. Identification and quantification of Ph positive metaphases have become valuable tools to confirm the diagnosis and to monitor therapeutic response (1,2). However, desire for further improvement in sensitivity has led to the introduction of molecular techniques for measuring BCR-ABL transcripts. Discovery of the ABL tyrosine kinase inhibitor, imatinib mesylate, was revolutionary in the treatment of CML and the only method to measure the level of remission accomplished is real time quantitative polymerase chain reaction (2,7,11). Measuring the level of BCR-ABL transcript, presented as log reduction in the BCR-ABL/ABL ratio, is used to enable better assessment of response of individual patients to treatment with IM and to evaluate the efficacy of a treatment protocol. It can also be used to stratify patients according to the risk of relapse and is crucial to revise therapeutic strategy (1,4).

Although complete estimate of therapeutic response according to the European Leukemia Net includes clinical assessment, blood count, cytogenetic revision of bone marrow and calculation of the BCR-ABL/ABL ratio every 3 months from therapy initiation (2), in this study only log reduction in the BCR-ABL/ABL ratio was used to monitor CML patients on the standard treatment protocol with IM. In general, reduction in the BCR-ABL/ABL ratio correlates closely with the increasing hematological as well as cytogenetic response. It is known that 2 log reduction in the BCR-ABL/ABL ratio in 12-month follow up is in concordance with Ph negativity in bone marrow (CCyR) (10). In this period, RQ-PCR becomes the only tool for further monitoring of molecular response, which depends on the rate and degree of BCR-ABL transcript reduction (2,11).

Patients in study group I achieved MMoR with detectable or undetectable BCR-ABL transcript during 2-year follow up and fulfilled the criteria for favorable long term prognosis according to literature data (10). Despite this, monitoring should be performed indefinitely, including patients with undetectable BCR-ABL transcript because of

način za daljnje promatranje molekularnog odgovora koji ovisi o stopi i stupnju smanjenja prijepisa BCR-ABL (2,11).
Bolesnici I. skupine odgovorili su na terapiju i bili su pozitivni ili negativni za prijepis BCR-ABL tijekom dvogodišnjih dolazaka na kontrolu i promatranja te su ispunili kriterije za povoljnu dugoročnu prognozu prema navodima iz literature (10). Unatoč tome, praćenje treba provoditi neograničeno dugo te uključiti i bolesnike s negativnim prijepisom BCR-ABL što je različito od potpune molekularne remisije jer je RQ-PCR nedovoljno osjetljiva metoda (2,11). Rezultati I. skupine pokazuju, da čak i kod takvih bolesnika, terapijski odgovor (MMoR) može izostati te mogu doživjeti recidiv zbog stečene otpornosti na IM ili zbog interakcija s drugim lijekovima. Jedan od bolesnika nije više odgovarao na terapiju u trenutku kad je počeo uzimati kompetitivni lijek (tamsulosin hidroklorid). Taj je bolesnik, kad je prestao uzimati tamsulosin hidroklorid, postao opet negativan za prijepis BCR-ABL. Teoretski, mehanizam reakcije mogao bi biti kompeticija s IM za prenosioce te posljedično tome stanična raspoloživost IM. Još je jedan bolesnik pozitivan za prijepis BCR-ABL, koji je pozitivno odgovarao na terapiju, doživio recidiv nakon 11 mjeseci, što predstavlja razlog više da ove bolesnike valja pažljivije promatrati, kako bi se mogao predvidjeti tijek bolesti i mogući recidiv.
Bolesnici iz II. skupine nisu reagirali na terapiju pomoću IM. Većini je CML dijagnosticirana godinama prije uvođenja IM u terapiju CML, pa su uzimali busulfan i interferon-alfa u različitim razdobljima, te stoga nisu reprezentativni za procjenu djelotvornosti liječenja. Za mali broj bolesnika iz ove skupine kojima je nedavno dijagnosticirana CML i kod kojih uzimanjem IM nije došlo do reakcije bio je, prema navodima iz literature, potreban drukčiji pristup: viša doza IM i drugih inhibitora tirozin-kinaze (10). Naši su rezultati pokazali da je povećana doza IM izazvala trenutan odgovor kod malog broja bolesnika, no niti jedan od njih nije dugoročno odgovorio na terapiju (Tablica 2.). S druge strane, uvođenje i upotreba druge generacije inhibitora tirozin-kinaze ABL, dasatiniba i nilotiniba, što ovisi o dokazanoj točkastoj mutaciji kod bolesnika otpornog na IM (s iznimkom mutiranog klona T315I), daje snažnu stopu povoljnog terapijskog odgovora (2). Rezultati dvaju bolesnika koji su reagirali na terapiju u manje od godinu dana nakon početka terapije, jedan dasatinibom, a drugi nilotinibom, potvrđuju djelotvornost ovih lijekova. Potaknuta su daljnja istraživanja kako bi se ispitala dugoročna djelotvornost ovih lijekova i moguće neželjene nuspojave. Da je presađivanje koštane srži još uvijek terapija prvoga izbora kod mladih bolesnika otpornih na IM s HLA-odgovarajućim darivateljem, potvrđuje slučaj bolesnika iz II. skupine. Taj bolesnik nije reagirao na terapiju IM, no nakon transplatacije je u godinu dana praćenja postigao i zadržao MMoR.

limited sensitivity of RQ-PCR which differs with complete disappearance of leukemia (2,11). Results in study group I indicated that even those patients could lose MMoR and relapsed due to acquired resistance to IM or to interactions with other drugs. One of the patients lost MMoR when he started using, what seemed to be, a competitive drug (tamsulosin hydrochloride). Incidentally, when this patient stopped using the same drug, BCR-ABL transcript became undetectable again. A hypothetical mechanism could be competition with IM for a transporter and consequently availability of IM in the cell. The other patient with MMoR and detectable BCR-ABL transcript relapsed after 11 months, a reason more why these patients should be more carefully monitored to predict the course of disease and possible relapse.
Group II patients never achieved MMoR with IM. Most of them were diagnosed with CML years before IM era and were pretreated with busulfan and interferon-alpha for different periods of time, and therefore they were not representative for evaluation of treatment efficacy. A small minority of newly diagnosed CML patients from this group that did not achieve MMoR with IM required different therapy approach according to literature, i.e. a higher dose of IM and other tyrosine kinase inhibitors (10). Our results indicated the increased dose of IM to provoke temporary response in a minority of patients but none of them achieved MMoR (Table 2). On the other hand, the use of a second generation ABL tyrosine kinase inhibitors, dasatinib or nilotinib, which depends on a point mutation found in an IM-resistant patient, produces high response rates, with the exception of T315I mutant clone (2). The results obtained in two patients that achieved MMoR in less than a year from therapy introduction, one with dasatinib and the other with nilotinib, confirmed the efficacy of these drugs. Additional studies are stimulated to test long term efficacy of these drugs and possible adverse events (3). That bone marrow transplantation remains first choice therapy for young IM-resistant patients with HLA-matched donor was confirmed by a patient from study group II. This patient did not respond to IM therapy but after transplantation maintained MMoR for almost a year.
If the same analogy from the last two groups is applied, further development could be predicted in group III patients, i.e. seven newly diagnosed CML patients with IM as first line therapy. Two of these patients reached more than 3 log reduction in 6 and 10 months, and the other two achieved more than 2 log reduction in 3 and 8 months. It is reasonable to expect for these patients to achieve and maintain MMoR. The last three patients had less than 2 log reduction in 6 months but it is still too early to predict failure despite their poor response to IM therapy.
In conclusion, the success of IM therapy for CML has led to recommendations based on published studies (1,2,6,7).

Ako se upotrijebi analogija dviju posljednjih skupina, može se zaključiti da bi se daljnji tijek bolesti mogao predvidjeti i za bolesnike III. skupine, za sedmoro bolesnika kojima je nedavno dijagnosticirana CML i kod kojih je primijenjen IM kao prva linija terapije. Dva bolesnika postigla su smanjenje više od 3 log jedan u 6, a drugi u 10 mjeseci. Druga dva su u 3, odnosno 8 mjeseci postigli smanjenje više od 2 log. Razumno je očekivati da ti bolesnici postignu i zadrže MMoR. Zadnja su tri bolesnika u 6 mjeseci imali smanjenje manje od 2 log, ali je još uvijek prerano predviđati neuspjeh, usprkos slabom terapijskom odgovoru na IM.

Zaključno, uspjeh terapije pomoću IM u liječenju CML potaknuo je izradu preporuka koje se temelje na objavljenim istraživanjima (1,2,6,7). Bolesnici kojima je dijagnosticirana CML, trebali bi proći početno liječenje pomoću IM (početna doza 400 mg na dan) i u slučaju pozitivne reakcije, terapiju treba nastaviti bez vremenskog ograničenja. Svi se bolesnici trebaju pratiti u određenim razdobljima, što uključuje izradu kliničke procjene, krvne slike, citogenetičke procjene koštane srži i mjerenja fuzijskog prijepisa BCR-ABL svaka 3 mjeseca, kako bi se na svakoj kontroli odredilo radi li se o osobi koja ne odgovara na terapiju pomoću IM i otporna je na IM. Bolesnici kod kojih terapija pomoću IM nema uspjeha ili koji više ne reagiraju na nju, moraju proći probir na mutacije kinaze ABL, kako bi se omogućila odgovarajuća promjena u terapiji. Uvođenje i upotreba druge generacije inhibitora tirozin-kinaze ovisi o točkastoj mutaciji otkrivenoj na genu ABL te se ne preporuča bolesnicima s mutiranim klonom T315I. Takvi bolesnici imaju mogućnost presađivanja koštane srži ili primjene kemoterapije (hidroksikarbamid, interferon-alfa ili citarabin).

Rezultati zabilježeni u ovom istraživanju potvrđuju da pridržavanje preporuka omogućuje optimalno liječenje bolesnika s CML.

Patients diagnosed with CML should receive initial treatment with IM (starting dose 400 mg *per* day) and, if they respond, should continue with therapy indefinitely. All patients should be monitored at defined time points, including clinical assessment, blood count, bone marrow cytogenetics, and BCR-ABL transcript measurement every 3 months to determine non-responders and IM resistance at each time point of the follow up. Patients who fail treatment with IM or lose response should be screened for the presence of ABL kinase mutations in order to ensure proper change in therapy. The use of second generation tyrosine kinase inhibitors depends on a point mutation discovered in ABL gene and is not recommended for patients with a T315I mutant clone. These patients have a possibility for bone marrow transplantation or use of standard chemotherapy (hydroxycarbamide, interferon-alpha or cytarabine).

The results obtained in this study confirmed that compliance with these recommendations ensures optimal treatment for patients with CML.

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