

NEW FRONTIERS IN MYELOID MALIGNANCIES –
TARGETED THERAPIES,  CLINICAL STRATEGIES

INFORMATION LETTER OCTOBER 2011

ABSTRACTS

ELN Frontiers Meeting

**TARGETED THERAPIES,
CLINICAL STRATEGIES:**

**Focus on
CML, AML and MDS**

Chairs: Michele Baccarani, Rüdiger Hehlmann

Co-Chairs: Francisco Cervantes, Gert Ossenkoppele, Theo de Witte

7 - 9 October 2011, **Berlin**, Germany

Editorial



Dear Colleagues

It is our pleasure to hold the ELN Frontiers Meeting 2011 entitled: New frontiers in myeloid malignancies – targeted therapies, clinical strategies in Berlin. Berlin is a city of culture, politics, media and science, well known for its urban settings and historical legacy.

Building on 5 years of educational excellence in hematological malignancies, the scope of the ELN Frontiers 2011 has expanded from chronic myeloid leukemia (CML) to acute myeloid leukemia (AML), myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPNs). The ELN Frontiers Meeting 2011 is dedicated to examining treatment advances, latest results from ongoing clinical trials, management recommendations and data on novel therapeutic agents in these diseases.

In the second decade of targeted therapy, evidence from trials with second-generation TKIs in front-line treatment of Ph-positive CML shows that responses can be achieved earlier compared to standard imatinib, leading to more ambitious treatment goals. The European Treatment and Outcome Study (EUTOS) for CML, now in its fifth year, continues to expand access to cutting-edge clinical support programmes and to educate European clinicians with regards to the latest disease management tools and techniques.

Significant advances have been made in the development of prognostic and diagnostic criteria of Ph-negative MPNs. New therapeutic compounds continue to be studied and developed. One pivotal contribution, the identification of JAK2 mutations, has increased our understanding of the molecular pathology of these disorders and prompted the investigation of JAK2 inhibitors in current clinical trials.

The ELN Frontiers Meeting 2011 program including this abstract book reflects on these topics and provides a space for discussions on further associated complex issues.

We are looking forward to this year's important leukemia event, to all presentations, discussions and experts exchange.

Yours sincerely

M. Bacarani and R. Hehlmann

Content

CHRONIC MYELOGENOUS LEUKEMIA

- | | |
|---|-----------|
| [1] Pregnancy management and outcomes in patients with CML receiving tyrosine kinase inhibitors | 4 |
| [2] K356dup – an in-frame insertion in the BCR-ABL gene in imatinib-resistant CML patient | 6 |
| [3] Role of molecular monitoring in treatment of CML – one center’s experience | 7 |
| [4] Tackling the statistical challenges arising from new endpoints in clinical trial on CML | 8 |
| [5] Do variants of BCR-ABL gene transcript influence relapse-free survival and rate of cytogenetic response in patients with CML on imatinib therapy? | 9 |
| [6] Ph-negative BCR-ABL-positive CML patients have higher resistance rate to imatinib treatment in comparison with Ph-positive ones | 10 |
| [7] Successful pregnancies in CML patients treated with tyrosine kinase inhibitors | 11 |
| [8] Quality of life in CML patients treated with imatinib – prognostic impact of baseline values | 12 |
| [9] Variant fusion signals detected by fish analysis in CML patients: Do they indicate poor prognosis? | 13 |

ACUTE MYELOID LEUKEMIA

- | | |
|--|-----------|
| [10] In vivo proliferation and apoptosis of leukemic cells during induction chemotherapy in patients with AML, pilot trial | 14 |
|--|-----------|

MYELOYDPLASTIC SYNDROMES

- | | |
|--|-----------|
| [11] Risk factors for progression of lower-risk MDS to AML | 15 |
| [12] Outcomes of patients with MDS/MPN with emphasis on MDS/MPN-unclassified | 16 |

OTHERS

- | | |
|---|-----------|
| [13] Treatment of systemic sclerosis with TKIs. Could be a new indication? | 17 |
| [14] Mutation screening of druggable target molecules can be readily introduced in the management and diagnosis of hematologic malignancies | 18 |

[1] Pregnancy management and outcomes in patients with chronic myeloid leukemia receiving tyrosine kinase inhibitors

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Background

Tyrosine kinase inhibitor (TKI) therapy has improved survival rate and quality of life for the patients with chronic myeloid leukemia (CML). Therefore pregnancy management in CML patients is an important issue. We should be informed about the outcomes and therapy risks on TKI therapy by analyzing the existing data.

Aim and methods

We continue to collect information about pregnancy outcomes in CML patients on TKI therapy (imatinib, nilotinib, dasatinib) from different clinics in the Russian Federation and also describe the cases observed at the Hematology Research Center, Moscow.

Results

The outcomes for 37 pregnancy cases in CML patients on imatinib therapy and about 5 pregnancies on second generation TKIs (in females and in partners of males) are shown in Table 1. There were favourable outcomes in all 13 pregnancy cases in partners of CML males on imatinib: 13 healthy infants. All the patients were in chronic phase (CP), imatinib dose was from 400 to 600 mg daily, no treatment interruption at impregnation.

24 pregnancy cases on imatinib treatment occurred in 22 females. Two of them had subsequent pregnancies: the 1st pregnancy ended with medical abortion at the beginning of imatinib treatment and at the 2nd one was planned during complete molecular response (CMR) and resulted in healthy infant delivery in both cases. 22 females were in CP CML, 2 in accelerated phase (AP) at diagnosis. Imatinib dose was from 400 to 600 mg daily. One woman had a treatment interruption at impregnation.

The general approach was to stop imatinib after pregnancy diagnostics. Individual decisions in pregnancy management highly depended on the CML status and were made for every case (Table 2). Females in CMR were monitored for minimal residual disease (MRD) and did not require any therapy. Supportive treatment was used in case of cytogenetic or hematologic relapse (interferon alpha, hydroxyurea). One woman refused proper follow-up and used irregular uptake of imatinib. One woman received imatinib during the whole pregnancy period, a decision taken by her and her haematologist. After delivery, the patients restarted imatinib immediately, 2 women continued treatment interruption for 1 and 3 months respectively, for safe breastfeeding.

5 pregnancies occurred in CML CP patients on second generation TKI therapy. One pregnancy in the male's partner on nilotinib ended with premature delivery. One pregnancy in female was diagnosed on imatinib + hydroxyurea therapy and the woman was switched to nilotinib since 10th week of pregnancy, the outcome was a premature delivery of a low-weight child (2600 g). Among 3 pregnancies on dasatinib therapy the 1st one resulted in medical abortion, the 2nd one was prolonged: dasatinib was stopped since 6-7th week and the patient continued on hydroxyurea since the 2nd trimester, a healthy infant was born at term. That woman has now a subsequent pregnancy on dasatinib again (ongoing, 25th week, dasatinib stopped from 6th - 7th week).

Conclusions

The outcomes of known pregnancy cases in CML males partners on imatinib were favourable. For CML females on imatinib the disease status at impregnation was the main point for the safe management. The data about pregnancy outcomes on second generation TKI therapy are very limited, patients should avoid pregnancy. A careful study of individual cases is needed to develop further recommendations.

Table 1. Pregnancy outcomes in CML patients on TKI therapy

Pregnancy outcome	Therapy				
	Imatinib		Nilotinib		Dasatinib
	Partners of males	Females	Partners of males	Females	Females
Delivery at term, healthy infants	13	11			1
Premature delivery		1 (death)	1 (hyperbilirubinemia)	1 (low-weight)	
Ongoing pregnancy		5			1
Spontaneous abortion		2			
Medical abortion		5			1
Total number of cases	13	24*	1	1	3

* Among 24 pregnancies on imatinib there were 2 subsequent

** Among 3 pregnancies on dasatinib there were 2 subsequent

Table 2. CML status and therapy during pregnancy in 10 females who delivered healthy infants.

№	CML status (response for therapy)		Therapy during pregnancy	CML status after the delivery and imatinib restarted (not in a 1 st day 1 st analysis after delivery)
	Before the pregnancy	During the pregnancy		
1	CMR	CMR	no therapy	MMR
2	CMR	no data	no therapy	MMR
3	CMR	lost CMR and MMR	no therapy	MMR restored
4	CMR	lost CMR and MMR, Ph ⁺ +2% (FISH)	no therapy	Bcr-Abl/Abl 0.4% (1 st analysis) CMR restored in 3 months after restarting imatinib
5	MMR	MMR	Imatinib 400 mg daily	MMR
6	MMR	cytogenetic relapse	Interferon alpha 3 ME x 3 times per week	Ph ⁺ +59%
7	MCR	no data	Interferon alpha 3 ME x 3 times per week	CHR
8	no CR, CHR	hematologic relapse	Interferon alpha 3 ME x 3 times per week +hydroxyurea from the 3rd trimester	Partial HR
9	no CR, CHR	hematologic relapse	irregular uptake of imatinib low doses, no proper follow-up	hematologic relapse
10	no CR, CHR	hematologic relapse, restored during Interferon alpha therapy	Interferon alpha 3 ME x 3 times per week	CHR

CMR – complete molecular response, MMR- major molecular response, MCR – major cytogenetic response, CR – cytogenetic response, HR –hematologic response, CHR –complete hematologic response, FISH – fluorescent hybridization in situ.

[2] K356dup – an in-frame insertion in the BCR-ABL gene in imatinib-resistant CML patient

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Introduction of tyrosine kinase inhibitors (TKI) into the therapy of chronic myeloid leukemia (CML) resulted in significant increase of patients' survival rate. Despite this, approximately 15% of the patients do not respond optimally to TKI therapy. One of the most frequent mechanisms of TKI resistance is the acquisition of point mutations in the sequence which encodes the BCR-ABL kinase domain (KD). Sometimes the insertion/deletion mutants in the BCR-ABL gene are observed. Their role in the TKI resistance has been neglected so far because all of them results in frameshift and premature termination of BCR-ABL's translation. We present the detection of an insertion mutation in BCR-ABL gene which does not abolish kinase activity.

A 22 year old woman was diagnosed with CML in the myeloid blast crisis phase. After one year of imatinib therapy, the patient achieved complete haematologic and cytogenetic remission. RQ-PCR revealed that she had almost reached major molecular response (MMR, 0.12% IS). Subsequent monitoring of transcript levels (4 months later and 14 months from the diagnosis) showed a 2-fold increase of the BCR-ABL/ABL ratio (0.24% IS). Thus, a BCR-ABL KD mutation study was performed and K356dup – a single codon insertion – was found.

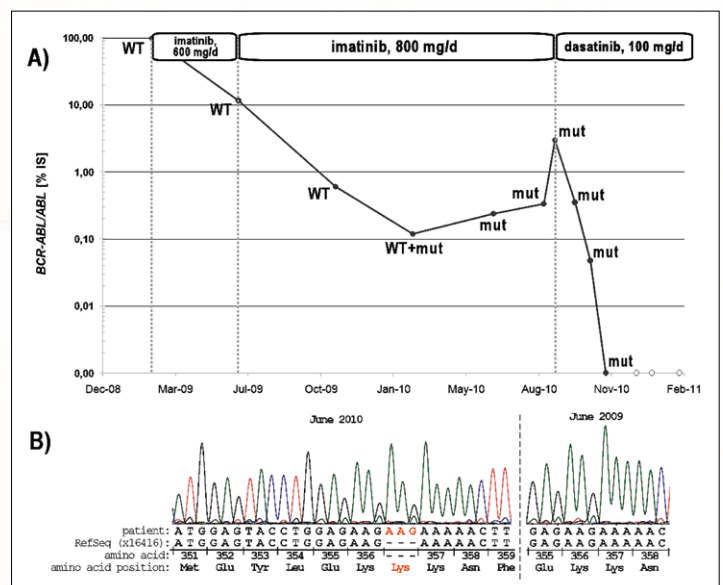
Total RNA was extracted from patient's peripheral blood using Chomczynski-Sacchi method. After reverse transcription, the sequence encoding BCR-ABL kinase domain was amplified and purified. The sequencing was performed using AbiPrism Genetic Analyzer 3100.

The sequence was analyzed with the SeqMan Pro software. The presence of an insertion was confirmed using Qiagel capillary electrophoresis system.

The presence of K356dup was confirmed in two separate analyses of two consecutive samples. The mutant allele was fully dominant – the potential native BCR-ABL sequence was below the detection threshold of direct sequencing. The RQ-PCR analysis of the subsequent sample confirmed a continuing increase in BCR-ABL transcript levels. Switching the therapy to dasatinib resulted in rapid reduction of BCR-ABL-positive cell population and MMR was achieved in less than 2 months. 112 days after introduction of second line treatment, the patient achieved complete molecular response.

The molecular mechanism of IM-resistance of BCR-ABL K356dup is probably

similar to that observed in the case of the M351T mutation and is related to abnormal autoregulation of the mutated kinase. It is known that the nearby tyrosine (Y353) plays a key role in binding the SH2 domain in BCR-ABL autoinhibition, and that the M351T substitution induces the kinase to an active conformation more frequently. The changes in the structure of the α -helix which surrounds Y353 limit its ability to interact with the SH2 domain. This impairs the inhibitory effect of IM, which binds only with the BCR-ABL kinase in an inactive conformation. K356dup is located too far away from the drug's contact regions, therefore direct interaction of the additional lysine with inhibitor molecules is rather unlikely. Our speculations seem to be correct, because administration of dasatinib in the 2-line therapy proved to be very effective. This drug is able to bind with both active and inactive conformations of BCR-ABL.



A) Patient's BCR-ABL transcript level and mutation status;
B) Sequence of BCR-ABL – with and without mutation.

[3] Role of molecular monitoring in treatment of chronic myeloid leukemia – one center's experience

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Background

Modern treatment of chronic myeloid leukemia (CML) in Ukraine is represented mainly by the tyrosine kinase inhibitor (TKI) imatinib, approved as front-line treatment for CML. Both at diagnosis and during CML course, cytogenetic and molecular investigations are the most reliable methods for disease monitoring with well-defined timing, although not always followed in Ukraine. Aim. The aim of this study was to analyze our own 5-year experience of managing CML patients treated with TKIs.

Methods

Of 89 CML patients monitored at our center, we selected a subgroup of 35 patients with complete cytogenetic response (CCyR). Discussed below are results of cytogenetic (35 patients) and molecular (15 patients) monitoring of CML in this subgroup including 17 women and 18 men (age 21-63 years), treated with imatinib or nilotinib. Cytogenetic response was evaluated at 6, 12, 24, and 36 months of treatment. Molecular investigation (quantitative polymerase chain reaction, qPCR) was performed in 15 patients with CCyR at 'GenoTechnologija' laboratory (Russian Federation), as in Ukraine this methodology was not available at the time of study.

Results

Among 89 CML patients treated at our center with imatinib front-line or as subsequent treatment, 35 achieved CCyR. In majority of non-responders, treatment failure could result from treatment interruptions due to drug non-availability or heavy pre-treatment with cytostatics. For this 5-year period 10 patients died due to disease progression. All of them never achieved CyR. In a subgroup of patients with CCyR, the initial dose of imatinib ranged from 400 to 800 mg. In case of suboptimal CyR, the dose of imatinib was either modified or patients were switched to nilotinib. At 3 months of imatinib therapy all patients achieved complete haematological response. At 6 months, CCyR was reached in 20 patients, major cytogenetic response (MCyR) in 13 and minor CyR in 2 patients. After 12 months CCyR was detected in 31 patients; MCyR in 2 patients; and 2 patients had minor CyR. At 24 months, 28 patients remained in CCyR; 3 were moved to nilotinib due to CCyR loss or absence of MCyR.

Molecular investigation was performed in 15 patients with CCyR. Complete molecular response (CMR) was achieved in 10 patients, major molecular response (MMR) in 4 patients. One patient failed to reach molecular response. Regular molecular evaluation was performed in 7 patients with CMR. Among these 3 had sustained CMR confirmed by repeated tests; loss of CMR was detected in 4 patients. Two patients with sustained CMR insisted on stopping treatment with TKI. One of them remains in CMR (12 months without treatment); in another one loss of CMR (0.14%) was detected after 6 months of treatment stop.

Conclusions

All patients achieving CCyR were treated with imatinib as first or second line treatment. The major problem in Ukraine remains initiation of TKI treatment in late phases of CML, due to limited availability of TKIs and unsatisfactory access to molecular monitoring of disease. Similar to international data, our own results suggest that early initiation of TKI treatment and regular molecular monitoring can contribute to achieving adequate results of therapy for CML.

[4] Tackling the statistical challenges arising from new endpoints in clinical trial on chronic myeloid leukemia

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Objectives and background

Due to the success of tyrosine kinase inhibitors in chronic myeloid leukemia (CML), significant differences between treatments in terms of overall survival (OS) will not be observed for a long time. Thus, the focus has changed to remission parameters which are regarded as surrogate markers for OS. To master the new methodological challenges involved, there is ongoing discussions by investigators as to the proper statistical analyses of these endpoints in CML.

Methods

For 'time-to-(first)-remission (TTR)' endpoints (e.g. first complete cytogenetic remission, CCR), the handling of competing risks like 'death before a possible observation of a first CCR' is discussed. It is outlined why it is rather advisable to investigate remission status at fixed time points. Furthermore, critical issues with regard to composite endpoints are considered.

Results and significance

A prerequisite for the application of Kaplan-Meier analysis is 'non-informative censoring' implying that the cause of censoring does not alter the probabilities to observe the investigated event. This means with sufficiently extended follow-up beyond a censored observation time, a first CCR (e.g.) will eventually be recorded. For the competing event 'death before CCR' this prerequisite of 'non-informative censoring' is not met. Instead, the probability to observe a first CCR is instantly reduced to zero. The adequate approach to investigate a TTR endpoint with the presence of competing events is the calculation of a cumulative incidence function.

However, the problem that the date of first remission is not exactly known still exists. The detection of a first CCR depends on protocol compliance and monitoring intervals. By contrast, the focus on clinically relevant fixed time points supports more complete data and leads to better interpretable results gained through easier understandable methods of analysis.

A composite endpoint 'failure-free survival (FFS)' might be defined as survival until one of four events is observed: Failure of achievement or loss of a certain remission status, disease progression, or death. With FFS, missing-value problems regarding the events linked to insufficient remission are likely and the different failure causes imply difficulties with interpretation. In contrast to progression and death, remission failures alone are much less critical.

Conclusions

TTR or complex composite endpoints as the primary endpoint of a clinical trial are not recommended. However, TTR analysis supports judgment on the velocity of drug response and on the time until a certain remission should be waited for. If validated as a surrogate marker, the remission status at such a time could be chosen as the primary endpoint. In accordance with accuracy and ease of interpretation, OS and progression-free survival are suggested as further primary endpoints. If regarded as (secondary) endpoints, TTR parameters and composite endpoints demand analysis of all (competing) events and caution with outcome interpretation.

Within the German CML Study Group, a consensus on the analyses of remission endpoints and principles of handling of complex composite endpoints was achieved (see Pfirrmann et al., *Leukemia* 2011 May 20 [Epub]). The next step is already underway: a multinational recommendation by the European LeukemiaNet providing the definition and a detailed discussion of future endpoints and their analyses in CML.

[5] Do variants of BCR-ABL gene transcript influence relapse-free survival and rate of cytogenetic response in patients with chronic myelogenous leukemia on imatinib therapy?

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Background

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder, characterized by the reciprocal translocation of t(9;22)(q34;q11) (Philadelphia chromosome), which results in a BCR-ABL fusion gene on chromosome 22. The vast majority of CML patients have the breakpoint in the chromosome 22 in the major breakpoint cluster region (M-bcr) between exons e13 and e14, also known as b2 and b3. The most frequently transcripts revealed are b2a2 and b3a2, which encode 210 kDa proteins. An influence of variants of BCR-ABL gene (b2a2 and b3a2) on course of CML has been widely discussed for several years. There are many conflicting data concerning prognostic value of transcripts' type.

Objective

To determine relapse-free survival (RFS) and cytogenetic response rate to imatinib treatment in patients with two variants of BCR-ABL transcript.

Methods

58 CML patients, median age 48 years (17-80), 28 female, 30 male, were treated with imatinib in hematological clinics of Saint-Petersburg and the north-west region of Russia since 2003. Diagnosis was established by detection of Philadelphia chromosome by conventional banding analysis or FISH in case of cryptic or variant translocation. Transcript variant of BCR-ABL gene was detected by reverse transcriptase-polymerase chain reaction (RT-PCR). RFS as incidence of CML-related death, disease transformation or loss of cytogenetic response was evaluated with Kaplan-Meier curves. Cytogenetic response was estimated by the percent of Ph-positive cells which were found in cytogenetic studies (30-50 metaphases). Rates of major cytogenetic response (MCyR) at 6 months and complete cytogenetic response (CCyR) at 12 months from start of therapy were end points of the study.

Results

RT-PCR revealed b3a2 variant in 30 (51.7%) patients, b2a2 - in 28 (48.3%). The median age of patients was 48 years (17 - 80) for patients with b3a2 transcript, and 49 years (26 - 72) for patients with b2a2. Expression of transcript variant b3a2 of BCR-ABL gene was frequently found in female group (19 from 28 [67.9%]), in comparison with male group (11 from 30 [36.7%]) ($p = 0.017$). There were no significant differences in RFS ($p = 0.38$) or MCyR rate at 6 months (14/19, 73.7% with b3a2; 12/17, 70.6% patients with b2a2; $p = 0.56$) and CCyR rate at 12 months of imatinib therapy (16/28, 57.1% patients with b3a2; 14/26, 53.8% patients with b2a2; $p = 0.51$) depending on transcript variant.

Conclusions

There were no significant differences in RFS and key cytogenetic responses to imatinib therapy between two transcripts (b2a2 and b3a2) detected at diagnosis in patients with CML.

CML

[6] Ph-negative BCR-ABL-positive CML patients have higher resistance rate to imatinib treatment in comparison with Ph-positive ones

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Objective

To evaluate imatinib (IM) treatment efficacy in Ph-negative BCR-ABL-positive CML patients in comparison with Ph-positive ones.

Methods

251 CML patients who received 400 or 600 mg IM once a day were included in the current study. This group was consisted of 181 patients in early chronic phase (CP), 65 patients in late CP and 4 patients in accelerated phase (AP). Chromosome banding analysis (CBA) and interphase FISH were performed at the time of diagnosis and at every 6 months of IM treatment. In Ph-negative CML patients CCyR were assumed as less than 1% of BCR-ABL-positive nuclei. RQ-PCR was done every 3-6 months. Detection of point mutations in the BCR-ABL TKD was performed by direct sequencing of RT-PCR products. According to ELN recommendations failures were defined as no CHR at 3 months, no CyR at 6 months, less than PCyR at 12 mo, less than CCyR at 18 months, loss of CHR, CCyR or MMR at any time during treatment, newly acquired BCR-ABL mutation poorly sensitive to IM (G250E, E255V/K, T315I), or progression to the AP/BC. In Ph-negative patients 6 months and 12 months time-points, when CyR and PCyR have to be estimated, were excluded from the analysis due to lack of direct correlation between percentage of BCR-ABL-positive nuclei and CBA data (N. Testoni et al, Blood, 2009). Failure-free survival (FFS) and overall survival (OS) were calculated.

Results

With respect to t(9;22)(q34;q11) detection by CBA at the time of initial diagnosis, all CML patients were divided into 2 groups: Ph-positive (n=244) and Ph-negative (n=7). In spite of presence of normal karyotype, all patients in the second group harboured BCR-ABL fusion gene revealed by FISH and RT-PCR. Groups were not significantly different in age at diagnosis, sex, Sokal risk group distribution, stage of disease at the time of IM treatment beginning, types of BCR-ABL fusion transcripts. Median time of follow-up was 43 months in Ph-negative group and 36 months in Ph-positive. Although 6 of 7 Ph-negative CML patients achieved CHR by 3 months, only 1 of them was in CCyR by 18 mo, that was significantly lower than in Ph-positive group ($p < 0.001$). Moreover, Ph-negative patients had high percentage of BCR-ABL-positive nuclei in BM both at 12 months (median 35%, range 1-60%) and at 18 months of IM therapy (median 49%, range 0-92%). Two Ph-negative CML patients progressed to AP and died subsequently. None of Ph-negative patients had BCR-ABL mutations, duplication or amplification. FFS in Ph-negative CML patients treated by IM was significantly lower than in 244 Ph-positive ones 0.14 ± 0.13 vs. 0.62 ± 0.03 ($p = 0.007$), while OS was comparable: 0.70 ± 0.15 vs. 0.85 ± 0.02 , ($p = 0.47$), respectively.

Conclusions

In our series, treatment outcomes in Ph-negative CML patients who received IM at a dose of 400 or 600 mg once daily were significantly worse in comparison with Ph-positive ones. However, dose escalation or switching to second-generation TKIs prevented further disease progression or CML-related deaths. Resistance in the observed group seems to involve BCR-ABL-independent mechanisms.

[7] Successful pregnancies in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors

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Background

Tyrosine Kinase Inhibitor (TKI) therapy has dramatically improved the prognosis and quality of life for the majority of patients with chronic myeloid leukemia (CML). Female patients who wish to conceive are currently advised to discontinue TKIs during conception and pregnancy due to teratogenic effects of the drugs.

Methods

Here we report information about pregnancy outcomes in CML patients on TKI therapy who stopped treatment to become pregnant and received alternative treatment during the period of conception and pregnancy, usually alpha Interferon (IFN) at low dose.

Result and significance

Patient 1: A 24 years old woman was diagnosed with CML in October 2002. She achieved optimal response with imatinib and in September 2006 stopped treatment to get pregnant and initiated IFN (3 MUI three times a week). After two month of stopping imatinib, she became pregnant and in September 2008 gave birth to a normal male. After delivery, treatment with imatinib was resumed. In January 2011 she gave birth to a normal girl following the same procedures. The patient always has maintained complete molecular response (CMR).

Patient 2: Diagnosed with CML in September 2007 at the age of 34 years. At the time of becoming pregnant she was taking dasatinib (due to sub-optimal response with imatinib). The patient also received IFN during conception and pregnancy, and remained in complete molecular remission (CMR), giving birth to a normal male with spontaneous delivery in July 2011.

Patient 3: Diagnosed with CML in September 2007, 31 years old. She achieved cytogenetic complete response with imatinib without molecular response. In September 2010 she lost cytogenetic and hematologic response, probably because of poor adherence to treatment (she had morbid obesity), and started treatment with Nilotinib (800 mg day at first, decreasing the dose to 600 mg/day because of adverse events). At 3 months she achieved CMR, and after 6 months of maintaining CMR, decided to become pregnant. She has currently stopped Nilotinib therapy and is receiving IFN treatment.

Conclusions

Although we maintain the recommendation that patients treated with TKIs should avoid pregnancy due to recognized teratogenic effects of the drugs and the risk of losing response after therapy discontinuation, in those patients who obtain optimal response (CMR or MMR), treatment with TKIs could be suspended to become pregnant. In these cases, BCR/ABL should be closely monitored. In our patients low dose IFN was administered during the period of prolonged cessation of TKIs in an attempt to maintain response. This therapeutic strategy appears to be safe for the newborn and the mother while maintaining the antileukemic response in CML patients.

[8] Quality of life in CML patients treated with imatinib – prognostic impact of baseline values

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Objectives and background

Introduction of targeted drugs and more thorough techniques of disease control revolutionised outcomes in chronic myelogenous leukemia (CML) patients. To date CML is not a lifetime-limiting disorder for the majority of patients. In these circumstances, not quantity, but quality of life (QoL) becomes the major endpoint of CML management. At present, few efforts have been made to assess and improve QoL in CML patients. No specific interventions are included in modern CML guidelines to improve QoL. The aim of the study was to investigate prognostic significance of baseline QoL values in CML patients treated with imatinib.

Methods

QoL data was obtained from validation study of FACT-BRM questionnaire that was carry out in 2001-2003. Analysis was conducted on data from 103 CML patients treated with imatinib. Patient population consisted of 53 men and 50 women with median age on imatinib start 43.7 years (18.8-65.5 years). They received pre-treatment with hydroxyurea, busulfan, interferon and various chemotherapies. QoL was assessed by self completion of Russian version of the Fact-BRM one month before, on days 1, 8, 28, and months 3, 6, 9, 12, 24 of imatinib treatment. Median time between CML diagnosis and imatinib initiation was 32.7 months (0.3-157.3 months). Stratification of patients was built on baseline QoL values.

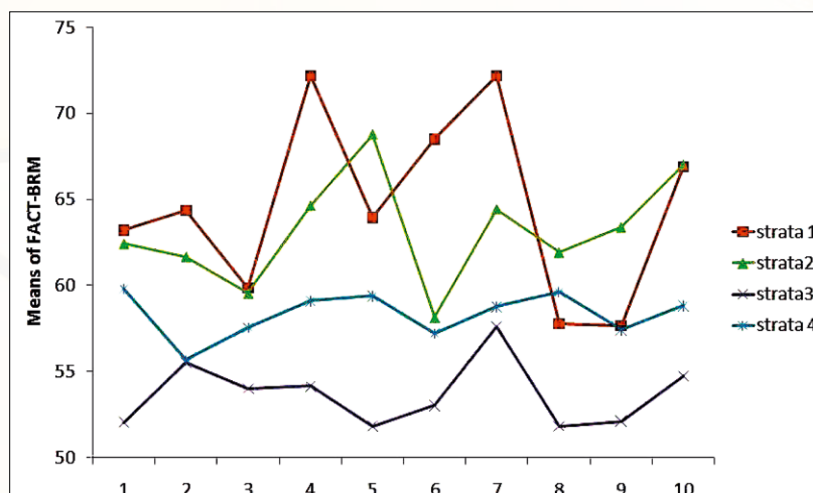
Statistical methods included cluster analyses by Ward's method, nonparametric Kruskal-Wallis ANOVA and Median test, and repeated measures ANOVA using Statistica 7.0 package. Missing items in questionnaires were processed with mean substitution.

Results

The rate of completed QoL questionnaires declined over time - 103, 99, 96, 92, 92, 88, 88, 86, 72, 57, 41 according to study points. A cluster analysis was based on baseline values and revealed 4 stratification groups with similar linkage distances (22, 19, 34 and 28 patients). These groups were not statistically differed by age or gender of patients, with the exception of female/male ratio in group 2. QoL profiles of all datasets were very similar to IRIS results. QoL changes according to stratification groups demonstrated statistically significant differences from baseline to final point in all scales with small exceptions (FACT-G in day 8, months 12, 24; emotional wellbeing in baseline, day 1; functional wellbeing in month 24; FACT-BRM in day 8). The composition scale – FACT-BRM profiles are presented in figure 1. Results of treatment in stratification groups did not reach statistical significance, but there were strong tendencies with respect to time of achievement of major molecular (p=0.13), complete molecular (p=0.32) response, duration of complete cytogenetic response (p=0.35), event-free survival (p=0.17). These findings could be influenced by the small number of patients in all groups and censored data.

Conclusions

QoL assumes more importance in treatment of CML patients. However the existing standard of care does not pay any attention to QoL management. Results of our QoL monitoring are similar to those obtained from some other studies. Baseline QoL values determine the profile of subsequent QoL and should be taken into account to personalize treatment strategy.



FACT-BRM means profile over time in stratification groups.

[9] Variant fusion signals detected by fish analysis in CML patients: Do they indicate poor prognosis?

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We evaluated sub-microscopic deletions of the ABL or BCR gene associated with t(9;22)(q34;q11) in chronic myelogenous leukemia (CML) using fluorescence in situ hybridization (FISH). The Vysis LSI BCR/ABL dual-fusion dual colour translocation probe was used on bone marrow aspirated cells of 90 patients with CML. Of these 90 patients, 14 patients (15.5%) showed variant fusion signals (VFS). Three patients were at age 21, 25 and 27. The rest ranged between 45-78 years. There were 8 females and 6 males. All patients except two had a high WBC count ranging between 50.000/mm³ and 329.000/mm³. In two patients WBC count was 27.000/mm³ and 34.000/mm³ respectively. Mean platelet count was 382.000/mm³.

Of these 14 VFS detected patients, all except one yielded "single fusion" (instead of two) and this single fusion was on chromosome 22 in 2/3 of the patients. Signal analysis based on metaphase chromosomes indicated that most of the relevant mutations were ABL gene deletions. In one patient a "triple fusion signal" was observed. One fusion signal was located on one chromosome 22, and the other chromosome 22 exhibited "double fusion" signals. Five patients showed both BCR and ABL gene deletions.

Cytogenetic analysis revealed the classical 46,XX,t(9;22)(q34;q11) or 46,XY,t(9;22)(q34;q11) translocation in 8 patients. One patient showed, 46,XX,der(9), two patients showed der(22) with no other visible chromosome rearrangements. One patient had a complex rearrangement involving chromosomes 3, 9, and 22 [46,XX,t(3q;9q;22q)]. In 2 cases no metaphase was obtained.

All patients were given imatinib as an initial therapy. After one year of therapy six patients were still not in CCyR and all were ABL deleted patients. One patient yielded MCyR and another one, CCyR and MMR. Four patients were recently diagnosed and complete hematologic response was achieved after 3 months of therapy. All four patients were both BCR and ABL gene deleted patients. No data could be obtained for one patient.

Although it is clear that more data for the clinical interpretation of variant Ph-positive CML mostly based on molecular work is needed, we suggest that variant Ph-positive CML could indicate a worse prognosis when ABL deletions or duplicate or triplicate fusions are present. It is not clear if deletions of both BCR and ABL genes could be included in the same category.

AML**[10] In vivo proliferation and apoptosis of leukemic cells during induction chemotherapy in patients with acute myeloid leukemia, pilot trial**

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Introduction

Proliferation studies in AML revealed that myeloid leukemia cells in most cases have low proliferation rate, with most cells in G0 phase. Various studies demonstrated that anti-leukaemic treatment induces apoptosis.

Objective

We analyzed changes in in vivo proliferation and apoptosis during induction anti-leukemic treatment and compared those findings with initial treatment outcome (CR/NR).

Materials and methods

We analyzed pathologically processed bone marrow particles from a cohort of 32 patients suffering from de novo AML, aged 45 yrs. All patients were treated with similar anti-leukemic treatment (ADE/MAE 26 pts; DA/MC schedule 6 pts). After obtaining informed consent, samples were taken at diagnosis and also at 3rd day (D3) from start of therapy (48h of cytotoxic exposure in vivo). We used commercial Ki-67 (MIB-1) antibody for proliferation studies and imaging kits (LSAB2, Dako) according to manufacturer prescription. Apoptosis was assessed on morphological level by counting cells with morphologically recognized apoptosis on at least 1000 cells and expressed as AI (%). Statistical analysis included parametric and nonparametric tests.

Results

Most of patients had myeloid AML (M0-M2 17 pts) while 12 patients had AML M4 and 3 patients M5 leukemia. Twenty one patient achieved CR (66%) and 11 were non responders after initial induction chemotherapy. Mean Ki-67 positivity was $8.4 \pm 9.3\%$ (0-47%). In 11/31 patients (35.4%) Ki-67 was absent, and in 38.8% was <10% of leukemic cells. In 8/31 patients (25.8%) Ki-67 was positive in >10% of leukemic cells. There was no correlation between percentage of Ki-67+ leukemic cells and morphology or cytogenetic features. Patients with tri-lineage dysplasia (11) had higher Ki-67 positivity (14 vs. 6.3%, MWU test $p < 0.05$). Initial mean AI was $3.0 \pm 1.59\%$. There was no difference in initial AI between patients according to leukemia type, tri-lineage dysplasia or karyotype (Kruskal Wallis $p > 0.05$). In samples after 48h of therapy we have found increase in Ki67+ in 22 pts but also decrease in 10 pts. Patients responding to induction chemotherapy (thus achieved CR) had significantly lower initial proliferation (CR Ki67+ 4.6% vs. 17.9% for NR, MWU $p < 0.05$). Significant changes were noted in 3rd day samples of patients achieved CR (Ki67+ $4.8 \pm 8.1\%$ increased to $7.2 \pm 6.6\%$) [Wilcoxon pair test $p = 0.05$] in comparison to NR patients (Ki67+ $17.9 \pm 26.7\%$ dropped to $10.8 \pm 13.4\%$). Patients with lower proliferation, Ki-67

labelled cells <10%, had better survival than others (log rank test $p = 0.06$). As expected, AI was increased to $7.77 \pm 4.5\%$ at D3 (Willcoxon paired $p < 0.01$). When we analyzed increase of AI and outcome we have found that patients in CR have significantly higher raise in AI at D3 than non-responders (AI $6.1 \pm 4.9\%$ vs. $2.3 \pm 1.9\%$, MWU $p < 0.05$).

Conclusion

Our results in a small cohort of patients in single center pilot trial confirmed that leukemic cells in AML are dormant, in most cases are non proliferative, similar to other findings. Patients with lower proliferation have better treatment outcome (CR achievement) and better survival. Patients able to increase in vivo proliferation and also in vivo apoptosis during treatment have better chances to achieve remission and to have better outcome. Thus, strategies to purge blasts into proliferation might improve outcome, and in vivo apoptosis measurement could help in determination of response before full recovery of patients.

[11] Risk factors for progression of lower-risk myelodysplastic syndromes to acute myeloid leukemia

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Objectives and background

Prognosis of patients with myelodysplastic syndrome (MDS) is very heterogeneous, particularly in the subgroup of lower-risk MDS (LR-MDS: Low/Int-1 IPSS or <10% bone marrow blasts). In addition, the rate of transformation to acute myeloid leukemia (AML) is not well established for LR-MDS as a whole group. The objective of this study was to analyse the rate of progression to AML, time to progression, and identification of factors which might influence on the risk of AML progression among LR-MDS patients.

Methods

353 LR-MDS patients from a single institution (1990-2010) have been analyzed. Median age was 72 years (17-93). Refractory anaemia (46%) and refractory anaemia with ringed sideroblasts (31%) were the most frequent FAB subtypes. 64/335 patients had bone marrow (BM) blasts 5-9%. 55% of patients had transfusion dependency (TD) at diagnosis or shortly after during follow-up. Karyotype was available in 106 patients according to IPSS (Low-risk: 43.3%. Int-1: 56.7%). To ensure that patients analyzed were LR-MDS as karyotype was not available in all cases, and considering that this missing data could make a change in IPSS stratification, patients with BM blasts 5-9% with no karyotype and 2-3 cytopenias were removed from analysis, assuming that intermediate or poor karyotypes would have categorized them as intermediate-2 or high risk. In case of karyotype defined

as good by IPSS, no change in IPSS score was possible even in case of 2/3 cytopenias with 5-9% blasts. Thus, 32 patients were removed from study by this consideration

Patients treated with 5-azacitidine, lenalidomide or allogeneic stem cell recipients were excluded from analysis as these interventions could possibly impact on survival or disease progression.

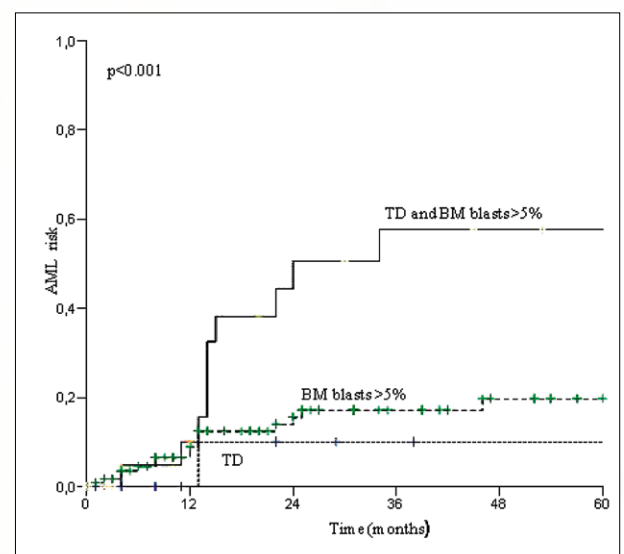
Results and significance

After 22 months median follow-up, AML progression occurred in 54 patients (15%) and 246/335 patients (69.7%) had died at last follow-up. Mean time to AML was 41 months. Variables that influenced on the risk of AML progression were TD (19% versus 10.5%, $p=0.05$) and BM blasts (32% versus 12%, $p=0.001$). By contrast, peripheral cytopenias, age and MDS subtype had no influence on the risk of progression to AML. Upon combining TD and BM blasts, 3 different subgroups of patients could be identified, with a rate of 12.5%, 14.4% and 40% of AML progression if BM blasts 5-9% ($n=16$), TD ($n=125$) or both ($n=30$) respectively. At 2 years, risk for AML progression was 9% for patients with BM blasts >5%,

8.5% for patients with TD, but this risk significantly increased up to 49.5% for patients with BM blasts >5% and TD (Figure 1, $p<0.001$). Mean time for transformation was 22, 19 and 13 months, respectively for each group. Overall survival for the group of patients who progressed to AML was <8 weeks.

Conclusions

Prognosis of LR-MDS is very heterogeneous. According to the current study, almost half of the patients with TD and BM blasts > 5% are at risk of progression to AML after 2 years from diagnosis. Thus, in spite of being classified as lower-risk, aggressive therapeutic approaches may be required in this subset of patients early after diagnosis.



MDS

[12] Outcomes of patients with myelodysplastic syndromes/myeloproliferative neoplasms with emphasis on MDS/MPN-unclassified

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Background

Data on outcomes of patients (pts) with myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPN), especially MDS/MPN-unclassified (MDS/MPN-U), are scarce.

Patients/methods

We retrospectively studied pts followed in our center, with MDS/MPN according to WHO 2008 criteria. Because of overlap characteristics of MPN and MDS, pts with systemic mastocytosis associated with MDS (SM/MDS) were also included. Pts with previous MDS or MPN were excluded. Response and disease progression were defined according to IWG 2006 criteria.

Results

Twenty-five pts with MDS/MPN were included. Median age was 70 y (range 19-79). Male/female ratio was 1.77/1. Diagnosis was CMML-1 N=7, CMML-2 N=7, JMML N=1, MDS/MPN-U N=8, systemic mastocytosis (SM)/MDS N=2, with one additional pt with CMML subsequently developing SM. At diagnosis, median WBC count was 18.8 G/L (range 3-120), ANC 15.5 G/L (0.6-70), monocytes 1.9 G/L (0.1-16), left shift 16% (0-28), Hb 11.2 g/dL (6-17), platelets 99 G/L (10-680), peripheral and bone marrow (BM) blasts 5% (0-17) and 7% (2-19), respectively (resp.). 25% of pts had platelets count \geq 400 G/L.

Splenomegaly, B-symptoms and BM fibrosis were present in 23%, 57% and 27% of pts, resp. Karyotype was fav, int and unfav in 55%, 36% and 9% of pts, with -7, +8, del(12)(p11), del(12)(q14;q21), +10, +21, and previously unreported t(9;12)(q13;q13) in 3, 6, and 1 pt each, resp., while +21 and i(17)(q10) appeared during disease progression other than AML transformation. IPSS was low/int-1 and int-2/high in 50% and 50% of pts, resp. JAK2 V617F and CKIT D816V mutations were detected in 2/6 pts and 2/2 SM/MDS pts, resp. 70% and 29% of pts were transfused at diagnosis with PRBC and platelets, resp.

Treatment included erythropoiesis stimulating agents (ESAs), low dose chemotherapy, intensive chemotherapy (IC) and azacitidine (AZA) in 40%, 36%, 16% and 48% of pts resp. Response rate to ESAs, IC and AZA was 60%, 14% and 14% resp. Response rate to AZA in CMML-1 pts was 33%. Dasatinib yielded no response in 1 SM/MDS pt with CKIT D816V.

3-year cumulative incidence of AML and median overall survival (OS) in pts with CMML-1, CMML-2 and MDS/MPN-U were 20%, 40% and 0 (P=0.059) and 39, 8, and 20 mo (P=0.50), resp. The pt with JMML died from AML transformation 3 months after diagnosis. 2/3 pts with SM/MDS died from disease progression w/o AML at a median of 10 mo after diagnosis. Median survival after dis-

ease progression other than AML transformation was 35, 15 and 14 mo in pts with CMML-1, CMML-2 and MDS/MPN-U, resp. (P=0.88). Cause of death was disease progression other than AML, AML transformation and unrelated to disease in 50%, 50%, and 0 and 80%, 0 and 20% of cases in CMML and MDS/MPN-U, resp. (P=0.10). Percentage of circulating blasts \geq 5% was the only independent factor affecting risk of AML transformation in the overall population (P=0.0004). Diagnosis other than CMML-1, WBC \geq 30 G/L, % of circulating blasts \geq 5% and IPSS high/int-2 were associated with worse survival in univariate analysis (P=0.06, 0.03, 0.04 and 0.08, resp.). No predictive factor of OS was found in multivariate analysis.

Conclusion

MDS/MPNs are heterogeneous disorders with respect to disease progression and AML transformation. MDS/MPN-U tended to differ from CMML-1 by shorter survival after disease progression other than AML, and from CMML-2 by lower risk of AML transformation. Mortality of pts with MDS/MPN-U was mainly attributed to disease progression without AML transformation. Alternatively to hypomethylating agents, therapeutic options in pts with MDS/MPN-U could include JAK2 inhibitors.

OTHERS

[13] Treatment of systemic sclerosis with tyrosine kinase inhibitors. Could be a new indication?

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In spite of prophylaxis, chronic graft-vs-host disease (GVHD) is the most common and late stage complication of allogeneic haematopoietic stem cell transplant (HSCT). Scleroderma is a rare form of chronic GVHD ascribed to abnormal reconstruction of the immune system. Generalized forms produce reduced mobility and almost 40% have extracutaneous affection. Conventional therapy with corticosteroids combined with other immunosuppressors have limited efficacy. Other options such as Psoralen-UV-A or extracorporeal photopheresis have variable effectiveness. Imatinib mesylate has recently been proposed as a therapy of interest for the treatment of systemic sclerosis related to its ability to inhibit c-Abl and platelet-derived growth factor receptor, tyrosine kinases involved in profibrotic pathways. The objective of this report is to present two cases of generalised scleroderma as manifestation of late GVHD after related allogeneic peripheral blood stem cell transplantation (PSCT).

Case 1

An 18 years-old female was diagnosed with T-Acute Lymphoblastic Leukemia. She underwent PSCT in September 2007. The preparative regimen was with busulfan and cyclophosphamide, and subsequently received cyclosporine over a short course of four days along with methotrexate to prevent chronic GVHD. No acute GVHD appeared after transplantation. Immunosuppression with cyclosporine and prednisone was maintained until day +240 post-transplantation and then gradually reduced until its suspension on day +480. Three months later she was attended to in the outpatient clinic for stiffness of small joints in hands, elbows and knees, accompanied by cellulites in lower arms. In addition, she presented with a waxy skin of the forearm attached to underlying tissues. These symptoms were compatible with scleroderma plaques according chronic GVHD. MRI images showed an inflammatory involvement of the fascia and septa interfascial with a wide infiltration. Elastography showed the impact only at the fascia, with no involvement of muscle fibres. The modified Rodnan skin score was: 48/60. She received combined therapy with mycophenolate mofetil, steroids and PUVA and 9 months later slight improvement was detected. We decided to change the strategy and start treatment with Imatinib mesilate 100 mg. It has been well tolerated, without any adverse effect, and after a 6 month follow-up, we have seen a progressive and substantial improvement in the hardening and stiffness of the patient's skin and joints.

Case 2

A 53 years old female was diagnosed with myelodysplastic/myeloproliferative neoplasm in November 2006. A PSCT was performed in October 2007. 22 months after PSCT, extensive skin plaques in dorso, abdomen and lower extremities with sclerosis, dyskeratosis and focal necrosis appeared. Elastography showed pseudonodular formations with severe abnormal stiffness in the subcutaneous tissue. The modified Rodnan skin score was: 50/60. She received combined therapy with mycophenolate mofetil, steroids and PUVA during 12 months without improvement. She then started therapy with imatinib mesilate 100 mg/d, with good tolerance.

Comments

Preclinical data using in vitro and murine models of fibrosis have demonstrated the antifibrotic properties of imatinib and other TKIs by inhibition of c-abl and PDGF. Whether imatinib will be tolerable or effective in the treatment of systemic sclerosis is the subject of several investigations.

OTHERS

[14] Mutation screening of druggable target molecules can be readily introduced in the management and diagnosis of hematologic malignancies

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Objectives and background

During the last 50 years, the somatic mutation theory of cancer has been the prevailing paradigm in cancer research and for hematologic malignancies, and a wealth of genomic aberrancies in bone marrow cells have been described. Some of these aberrancies such as the Bcr-abl translocation have been taken to in depth biological and pharmacological studies that have provided clinicians with a therapy options that targets the aberrantly activated tyrosine kinase with a new generation of specific inhibitors, thus hitting the presumed Achilles heel of CML. From the latter story we can learn that it is a long way going from the identification of a recurrent genomic aberration, analysis of its biological function, designing and testing a specific inhibitor and developing a manageable drug. Recent emphasis on the design of agents with inhibitory action on cellular pathways through inhibition of specific molecules seem to claim a paradigm shift from 'searching for the drug that hits the aberrant molecule' to 'identifying the patients that carries an aberrant molecule for which new agents are available'. Examples of relevant inhibitors of aberrantly activated molecules in hematologic malignancies are JAK2 and RAS-pathway molecules.

Methods

High through put screening of mutation hotspots in KRAS using amplicon Next Generation Sequencing (NGS) 454 technology (Roche Applied Science) provided instant information on activating mutations and of mutational load (percentage of mutated sequence reads) in DNA of hematologic samples. Gold standard Sanger sequencing was used to validate mutations revealed by NGS.

Results

Mutation analysis of AML, JMML, CMML and CML patient samples revealed a high number of activating KRAS mutations with mutation load varying from 44 to 0.1%. Samples with a mutation load of >10% were all found to be concordant when analyzed by Sanger sequencing and no mutations were found by Sanger sequencing in samples that were mutation 'free' by NGS 454 technology.

Conclusions

NGS 454 amplicon sequencing technology is a robust technology that reliably identifies somatic point mutations in KRAS with a sensitivity of at least 0.1%. Activating RAS mutations prevents the hydrolysis of RAS-GTP and results in constitutive activation of the RAS protein, enhancing cell survival and proliferation. The impact of KRAS mutations, in particular small KRAS mutated clones, on the course the disease remains to be demonstrated. In principle, the information on the KRAS mutation could be used by clinicians to contemplate the use of specific inhibitors of the RAS-pathway in combination therapies. Sensitivity of up to 10⁻³ (0,1% of mutated sequence reads) deems the 454 amplicon sequencing approach to be suitable for mutation screening during treatment follow-up of patients, and the 'open' design of 454 amplicon mutation screening favours screening of several mutations in parallel and the on-time addition of new samples for screening.

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