NEW FRONTIERS OF MYELOID NEOPLASIAS

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October 11-13, 2013, Prague, Czech Republic
Dear colleague,

we are delighted to welcome you to the 2013 ELN Frontiers Meeting titled New Frontiers of Myeloid Neoplasias. This official meeting of the European LeukemiaNet (ELN) is taking place on October 11-13 in Prague, Czech Republic. The meeting is hosted by the ELN and cosponsored by the University of Heidelberg and the Czech Leukemia Study Group for Life.

The ELN Frontiers meeting draws together a wide variety of experts in hematology from the greater European region to encourage valuable discourse on the pressing problems associated with chronic myeloid leukemia (CML), myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), and to discuss and share the newest developments in targeted treatment, patient management and patient outcomes.

In CML, imatinib and now second-generation TKIs have enabled great advances over the last decade in management and treatment – so much that we are now looking at treatment-free remission as an achievable goal for many patients; the recently updated ELN recommendations for the management of CML highlight this with renewed focus on the speed of achievement and the depth and persistence of response. Advances in diagnosis and monitoring, new breakthroughs in the understanding of molecular pathogenesis, and exciting results from clinical trials are helping to improve outcomes for MDS and AML patients. The recent inclusion of Janus kinase inhibitors amongst the treatment options for MPN patients has already led to significant improvements in quality of life. The ELN Frontiers meeting curriculum focuses on the recent advances in treatment, molecular and diagnostic tools, clinical management issues and promising clinical research in these disease areas.

Please join us on the poster walks which recognise and disseminate the contribution of researchers. Accepted poster abstracts may be found in our special edition of the ELN Newsletter and many will be presented orally. Complementing this outreach to new knowledge is our special research news session summarising recent highlights, both scientific and clinical, of preeminent experts and established researchers in these fields.

The ELN Frontiers Meeting builds upon seven years of education innovation in myeloid neoplasias. Please actively participate in this exciting congress with a rich mix of plenary and interactive parallel sessions including “meet the experts”, clinical case discussions, debates, poster walks, current news and oral presentations.

Yours sincerely

M. Baccarani and R. Hehlmann
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CML

[1] Targeted Next-Generation Sequencing for the identification of genomic BCR-ABL1 fusion junctions to quantify residual disease in CML patients in CMR

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Objectives and background
Recent studies indicate that 40% of CML patients who achieve complete molecular remission (CMR) on imatinib remain disease-free after drug discontinuation, raising the possibility of an “operational cure”. However, the safe introduction of a TKI withdrawal policy would require a reliable and cost-effective method of identifying patients with the lowest likelihood of relapse, which is likely to be related to presence of residual disease. Preliminary data suggest that PCR of genomic DNA might be more sensitive for the detection of residual disease than one that relies on cDNA and may therefore help to predict outcome post-withdrawal. However, the former method is arduous since it requires a customised patient-specific assay.

Methods
Here, we describe a method based on targeted-next-generation sequencing (NGS) allowing identification of BCR-ABL1 breakpoints from enriched genomic BCR and ABL1 DNA followed by rapid generation of DNA-based qPCR assays. The location of the BCR-ABL1 fusion junction was mapped in disease samples from 36 CML patients using Illumina’s MiSeq platform. A custom TruSeq DNA target enrichment kit (Illumina) was used to enrich for the BCR and ABL1 genes. The enriching probes were designed via the online tool Design Studio covering both genes plus 50kb upstream and downstream of BCR and ABL1, respectively. The workflow involved sample quantification, library prep, multiplexed sample pooling, enrichment-probe hybridisation, template preparation, and sequencing. Subsequent mapping of (t9,22) translocation junctions was performed via a custom designed bioinformatics algorithm.

Results
All breakpoints were successfully mapped. DNA qPCR assays were designed and validated for 18 patients. In clinical samples from patients in complete molecular remission, the RT-qPCR assays detected residual disease in 6 out of 18 patients (33.3%), demonstrating that DNA-qPCR can detect residual disease in patient samples in which CML cells persist below the detection threshold of RT-qPCR. Furthermore, we investigated disease status in 48 CMR samples coming from 6 CML patients (3 positive and 3 negative by DNA-qPCR) using digital PCR (dPCR) (Fluidigm BioMark HD platform) which allows single DNA molecule detection. We found that all 6 patients had detectable disease signals and that the level of positivity was reducing over time despite being negative by DNA-qPCR.

Conclusions
In conclusion, NGS-facilitated DNA-dPCR may therefore prove valuable for the stratification of patients with low levels of residual disease and, therefore, in the identification of patients for whom TKI therapy could be safely reduced or stopped.

[2] Arab Leukemia Net (ALN) registry and data base for chronic myeloid leukaemia (CML) in North Africa & Middle East (AFME) region. A call for action and declaration of intent

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Introduction
Reliable epidemiological information on Ph/BCR-ABL-positive CML is either sparse, lack ethnic diversity, adequate patients’ numbers or reliable data. This is particularly important in the recent TKI era for the Middle East & North Africa region including most of the Arab world. CML incidence rates in Western countries vary from 0.6 to 2.0 cases per 100,000 inhabitants, increase with age, and occur more often in men than in women. Geographic, industrial and environmental or ethnic variations might contribute to the variability of incidences of CML among registries. The prevalence rate has recently increased by use of TKIs. In clinical practice, some CML management areas are not in-line with the current recommendations. Problematic areas are sub-optimal timing of treatment decisions under monitoring, and unawareness of new molecular monitoring techniques and of beneficial new TKIs. Median age differs between cancer registries and clinical trials by 10-20 years. Reports of clinical studies underestimate the true age of the CML population. Elderly CML patients are underrepresented in clinical studies and thus have a reduced access to investigational therapies. Significant reduction in incidence of CML in recent periods was reported from India. The data of CML have to be observed for another decade to witness reduction in mortality because of changes in treatment management. The Arab world constitutes 22 countries that occupy the Middle East, North Africa and Asia, with a diverse ethnic population of 370 million inhabitants, and, marked socio-economic, geographic and gender differences, and most importantly, an exceedingly variable access to medical services. Little is known about burden of CML in Arab countries. There is a recent interest to observe incidence and mortality because of advent of new diagnostic and treatment policies for CML. Some clinical trials underestimate the age and incidence of CML, others suggest female CML patients present with more significant adverse per-treatment prognostic factors compared to men, yet achieve comparable outcomes. Hispanic patients present CML with lower risk profile and achieve better treatment responses compared to non-Hispanics. Thus the need to build up a credible data base/patient registry program for CML patients across AFME.

Objectives
To build a reliable data base and free information/support services, to raise awareness of need for improvement, and to design and implement a regional model/protocol for efficient and equitable delivery of CML healthcare in the AFME region.

Methods
We established a web-based portal for data input for all Arab countries...
The importance of ethnicity, socio-economic and gender differences in relation to disease incidence, diagnosis, and prognosis has been realized. Differences in these areas have become a major health policy focus. We examined the demographic and clinical features of CML patients presenting at Medical Centers in the Middle East/North Africa region by analyzing data reported to the ALN web portal. The study demonstrates that age-specific rates for CML are highest in the age group of 30-34 years, which is much lower compared to Western populations. No gender difference was found. PFS were equal in female and male patients.

Conclusions

In the last decade the importance of ethnicity, socio-economic and gender differences in relation to disease incidence, diagnosis, and prognosis has been realized. Differences in these areas have become a major health policy focus. We examined the demographic and clinical features of CML patients presenting at Medical Centers in the Middle East/North Africa region by analyzing data reported to the ALN web portal. The study demonstrates that age-specific rates for CML are highest in the age group of 30-34 years, which is much lower compared to Western populations. No gender difference was found. PFS were equal in female and male patients.

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Acknowledgments to Michele Baccarani

Results

Patients mean age at presentation was 41y (40y for males, 41y for females). The age specific rates were highest for the age group of 30-35 years. At diagnosis, 84% patients were in chronic phase CML, 9.1% in accelerated, and 6.9% in blast phase. Results of Sokal score were: Low-risk 55.8%, intermediate-risk 24.5%, high-risk 17.7%, and unknown 2%. EURO (Harford) score (56.3% low-risk, 18.4% intermediate-risk and 13.6% high-risk, while 2% unknown). Female patients presented with lower hemoglobin, higher platelet counts and smaller spleen size (p<0.0001). BCR-ABL transcript level analysis was performed to 96.6% of cases, cytogenetics by FISH to 77.6%. All patients received TKI therapy (61% imatinib, 24% nilotinib and 15% dasatinib). 96% of patients achieved hematologic response, 87% achieved PCR, 64% achieved CCyR, and 60% MMR within a period of 4 years follow-up.

The proportional female rate was 14% for female and 22% for male patients. Median survival/progression-free survival was equal in female and male patients.

[4] The prognostic value of BCR-ABL1 transcript type in chronic myeloid leukaemia patients treated frontline with nilotinib

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The baseline characteristics of the 2 groups were similar (no significant differences between groups were tested using X2 test, Fisher exact test or t-test, as appropriate. Response monitoring: conventional cytogenetic examination (bone marrow) and qPCR (peripheral blood). Definitions: MMR: BCR-ABL ratio <0.1% (International Scale); failures: according to 2013 ELN recommendations; events: failure or treatment discontinuation for any reason. The time-to-response and the outcome were estimated using the Kaplan-Meier method, and compared by log-rank test.

Results and significance

The baseline characteristics of the 2 groups were similar (no significant differences in age, Sokal/Hasford/EUTOS score distribution, clonal chromosomal abnormalities in Ph+ cells, NIL dose), except for the percentage of basophils in the peripheral blood, higher in patients with e13a2 transcript (3.4% vs 2.3%, p=0.01). The median observation was 43 months (range 18-69). The CCyR and MMR rates at 12 months were comparable in the 2 groups. The time to MMR was longer for patients with e13a2 transcript (6 months vs 3 months, p=0.04). The probability of overall survival (OS), progression-free survival (PFS) and failure-free survival (FFS) was comparable in patients with e13a2 and e14a2 transcript.

Conclusions

In our experience, based on 201 early CP CML patients treated frontline with NIL with a minimum follow-up of 18 months, the BCR-ABL transcript type did not show any relevant prognostic impact. The time to MMR was longer in patients with e13a2 transcript, but no outcome differences have been observed so far. The number of observed events was low and a longer observation is required.
**CML**

**[5] Regulation of BCR-ABL via its 3’ untranslated region**

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Objectives and background

The BCR-ABL fusion gene is the underlying cause of chronic myeloid leukemia (CML). This gene is formed by a reciprocal translocation between chromosomes 9 and 22. Therefore, BCR-ABL transcription is driven by the BCR promoter, whereas post-transcriptional regulation is likely controlled by the ABL1 3’ untranslated region (3’UTR). BCR-ABL “dosage” has been linked to disease phenotype, but it is unknown how the BCR-ABL gene is regulated. One of our aims is to examine how the 3’UTR controls post-transcriptional regulation of BCR-ABL. In particular, we seek to identify non-coding RNAs and RNA-binding proteins which exert regulatory effects through the ABL1 3’UTR.

Methods

Luciferase and GFP reporters for the ABL1 3’UTR have been utilised to determine the consequence that the ABL1 3’UTR has on gene expression. This system has also been used to map regulatory sequences within the 3’UTR and screen for putative regulators. RNA-EMSA experiments coupled with LC-MS/MS have been used to identify putative 3’UTR-protein interactions.

Results

Cloning the ABL3’UTR downstream of a reporter gene demonstrated that the 3’UTR is repressive. Insertion of a premature pol-ly-adenylation site in the 3’UTR rescued reporter expression, which is indicative of the 3’UTR acting via post-transcriptional control. Subsequently, serial deletions of the 3’UTR identified discrete regions with strong regulatory activity. We are currently investigating putative ABL1 3’UTR targets generated from prediction software and published data. In addition, we are developing a non-biased approach to pullout both RNA-binding proteins and microRNAs that interact with the BCR-ABL 3’UTR. This involves tagging the BCR-ABL 3’UTR via biotin or RNA-aptamers, followed by in vitro or in vivo isolation of RNA and protein which binds to the 3’UTR.

Conclusions

We have shown that the BCR-ABL 3’UTR is repressive and contains regions with negative regulatory elements. With the repression involving post-transcriptional control, current work is focused on identifying mi-croRNA and RNA-binding protein interactions with the 3’UTR. Unravelling of the mechanisms regulating BCR-ABL expression will contribute to a better understanding of the biology of CML and pave the way for innovative forms of targeted therapy.

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**[6] Sensitivity, reproducibility and clinical utility of next-generation sequencing (NGS) for BCR-ABL1 kinase domain mutation screening: Results from the CML Work Package of the IRON-II (Interlaboratory RObustness Of Next-Generation Sequencing) International study**

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Background and aims

In CML and Ph+ ALL patients resistant to tyrosine kinase inhibitors (TKIs), the BCR-ABL1 mutation status is an essential component of the therapeutic decision algorithm. Capillary Sanger sequencing (SS) is currently the gold standard for mutation screening of the BCR-ABL1 kinase domain (KD), despite key technical limitations including limited sensitivity. Benchtop next-generation sequencers have recently been introduced as potential diagnostic platforms, and there is growing interest in their clinical application. In the framework of the IRON-II (Interlaboratory RObustness Of Next-Generation Sequencing) international consortium, 10 laboratories from 7 countries (Italy, Germany, United Kingdom, Spain, Austria, Turkey, Czech Republic) have engaged in the set-up, standardization and validation of a laboratory-developed screening assay for BCR-ABL1 KD mutations based on the Roche 454 amplicon deep-sequencing technology.

Methods

Fusion primers were designed to generate four partially overlapping amplicons by nested reverse transcription (RT)-polymerase chain reaction (PCR), the first amplification step needed to select for the translocated ABL1 allele. Fusion primers were barcoded with multiplex identifiers (MDs) consisting of 10-base pair tags allowing multiplexing of 12 clinical samples in a single NGS run. The assay was designed in a ready-to-use 96-well plate format containing lyophilized oligonucleotide primers.

Results

Sequencing runs generated an average of 96,138 reads (range, 59,459-151,335). For the primer design selected for further evaluation, the coverage per amplicon ranged between 1,449 and 5,997 sequencing reads. To explore the sensitivity and accuracy of the assay, serial dilutions of BaF3 cell lines harboring four different known mutations (Y253F, E255K, T315I, M351T) into an unmutated BaF3 cell line (50%:50%, 25%:75%; 10%:90%; 5%:95%; 2%:98%; 1%:99%) were sequenced in parallel in two distinct laboratories (Bologna and Jena). In both centers, results showed a high linearity of mutation calling and accuracy of mutation detection and quantitation over the entire range of dilutions, down to 1% mutation abundance. Intra-run reproducibility and inter-run reproducibility were confirmed by a series of experiments in which a set of samples was re-sequenced in the same and in independent runs, respectively, with and without repetition of the RT and PCR steps. Importantly, we demonstrated that reproducibility could be maintained over a wide dynamic range of amplicon coverage (from 100 to 5,000 independent sequencing reads). A total of 554 clinical samples (2,216 amplicons) were analyzed by the 10 laboratories - including 517 clinical samples analyzed in parallel by NGS and SS and 30 clinical samples analyzed in parallel by NGS, SS and conventional pyro-sequencing. 394 of 398 (99%) variants detected by SS were also detected by NGS. In addition, comparison between NGS, SS and con-
[7] Minor subclones harboring small insertions and deletions probably due to aberrant splicing can frequently be detected by deep sequencing of the BCR-ABL kinase domain

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Objectives and background
The BCR-ABL kinase domain mechanisms that confer resistance to tyrosine kinase inhibitors (TKIs) in Philadelphia-positive (Ph+) leukemia is quite heterogeneous. We took advantage of deep sequencing (DS) to better characterize the spectrum of insertions and deletions in CML and Ph+ acute lymphoblastic leukemia (ALL) patients with response or resistance to TKI therapy.

Methods
A total of 110 samples of 41 CML and 16 Ph+ ALL patients who received one or multiple lines of TKI therapy were analyzed. DS was performed on a Roche GS Junior instrument that allows reliably detecting and characterizing deletions or insertions with a lower detection limit of 0.1%. In order to reconstruct the dynamics of evolution of these sequence variations in relation to the TKI administered and to the level of response achieved, we evaluated their presence in serial follow-up samples collected during TKI therapy in 15 patients.

Results
DS revealed a 35-base pair (bp) insertion in 35/41 (85%) of CML and 14/16 (87%) of Ph+ ALL patients. This sequence variation, already reported in the literature as ‘35INS’, consists of the retention of 35 nucleotides (nt) from intron 8 at the exon 8 to exon 9 border. It leads to a truncated BCR-ABL variant having 10 amino acids (a.a.) encoded by intron 8 sequences but lacking 653 C-terminal a.a., including 22 a.a. of the KD, along with the entire C-terminal region. 35INS was detected with variable abundance (range 0.1%-96% of all BCR-ABL transcripts), but in only three samples abundance was higher than 15-20% - thus detectable also by conventional sequencing. Re-sequencing a set of samples in the same and independent runs confirmed the presence of the 35INS and demonstrated that this variant was not a PCR or sequencing artifact. Longitudinal analysis showed that the expression of 35INS fluctuated over time with no apparent correlation with response levels. In addition, DS detected one in-frame deletion in 20/41 (48%) of CML patients and 7/16 (44%) of Ph+ ALL patients, with an abundance ranging from 0.2% to 19%. This previously unreported variant consisted of a 72bp deletion (nt.1233-1304) at the junction of exon 6 to exon 7, which causes the loss of 24 residues (a.a. 359-383) of the KD.

Conclusions
Our results further underline that DS technologies allow more accurate sequence characterization in comparison to conventional methods. Minor clones harboring insertions or deletions (always involving intron/exon junctions - which implicates alternative or aberrant splicing mechanisms) were found to be very frequent both in CML and in Ph+ ALL patients but, apparently, did not correlate with response or resistance to TKI therapy. In line with our findings, a very recent functional study has demonstrated that the truncated BCR-ABL protein resulting from the 35INS is kinase-inactive and should not play any role in TKI-resistance - in contrast to what had initially been hypothesized. Although this insertion does not predict for a specific TKI-resistance, its role in Ph+ Leukemia merits additional studies, and further analysis of a larger number of samples will be needed to better understand its biological and clinical relevance.

Support

[8] Nilotinib as first-line and salvage therapy for CML patients. A single-center experience

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Objectives and background
Nilotinib is a second-generation tyrosine kinase inhibitor approved since 2011 in Spain for the therapy of chronic-phase chronic myeloid leukemia on first-line, showing faster and deeper responses than imatinib.

Aims
To analyze our experience with nilotinib in the therapy of CML patients.

Methods
A retrospective chart review was performed using the institutional pharmacy registry including all CML patients who have received nilotinib between Jan-2007 to May-2013. General and clinical data, response to nilotinib at 3 months, side effects and use of others TKIs were registered.

Results
Nineteen patients were included, mean age: 60.3 y.o. (33-84), M/F: 10/9. Indication of use: first-line: 5, resistance to imatinib/dasatinib: 6 and intolerance to imatinib/dasatinib: 8 patients. Sokal: low: 11, intermediate: 7 and high: 2. One patient was in blast crisis, the rest on chronic phase. 8 patients were exposed to Hydrea +/- Ara-C before TKIs. According to 3-months response: 5/5 of first-line patients achieved < 9,5% of transcripts, from intolerance cohort: 2 patients improved the response (both in sub-optimal molecular response – soMR-), 3 achieved major molecular response (MMR) and 3: 4.5-log-MMR as second-line patients. Of the patients under nilotinib, 6 on CMR, only one is in CCyR. Of the patients under other therapy: 2 are in progression disease, 3 in MCyR, only one is in CCR. Of the patients under other therapy: 2 are in progression disease, 3 in MCyR and 4 in MMR.

Conclusions
Nilotinib permits to achieve deeper responses at 3 months of therapy in first-line as in second-line, and these responses are sustained; it is seems that probability the better results are related to early use/switch. No mutations were developed under nilotinib therapy. Dermatologic and digestive intolerance were the most registered.
Objectives and background

The aim of the present study was to analyze the response rates and outcomes in an independent cohort of newly diagnosed chronic myeloid leukemia (CML) patients treated frontline with nilotinib-based regimens. Nilotinib is a potent and selective BCR-ABL inhibitor approved for the frontline treatment of CML based on the results of the phase III ENESTnd study. The latest update (4-year follow-up) of this study demonstrated sustained superiority of nilotinib vs. imatinib (Hochhaus et al., EHA 2013, abstract 712). Outside of company-initiated trials, the CML Italian registry of nilotinib is the largest series of patients treated frontline with nilotinib-based regimens, representing an important resource for an independent evaluation of such patients.

Methods

The CML Italian registry of nilotinib includes 215 patients, enrolled in 2 multicenter phase II studies conducted by the GIMEMA CML WP (ClinicalTrials.gov NCT00481052 and NCT00769327) or treated at the Bologna University Hospital, with nilotinib 300 mg or 400 mg BID as initial treatment; 123 patients received a sequential treatment with nilotinib and imatinib, with a 3-months rotation period. The median age was 53 years (range 18–86). Ten out of 215 patients (5%) had a high EUTOS score. The median follow-up was 43 months (range 18–69 months). We analyzed the rates of complete cytogenetic response (CCyR) and major molecular response (MMR); the overall survival (OS; any death included), progression-free survival (PFS; progression to accelerated/blast phase [AP/BP] and deaths for any cause), failure-free survival (FFS; events: failures, permanent discontinuation of nilotinib for any cause, including deaths).

Results

The cumulative rates of CCyR and MMR were 93% and 88%, respectively. Of the evaluable patients, 176/189 (93%) were in partial cytogenetic response at 3 months; 185/199 (93%) were in CCyR at 6 months; and 138/190 (73%) were in MMR at 12 months (optimal responders according to ELN 2013 recommendations). Overall, 8 (3.7%) patients progressed to accelerated/blast phase [AP/BP], and all patients subsequently died (after a median of 13 months, range 1–34 months). All progressions occurred during the 1st year of therapy. Nilotinib-resistant mutations were identified in 5 of these patients (4 T315I; 1 Y253H). No difference in the rate of progression to AP/BP was observed between patients receiving nilotinib alone or nilotinib and imatinib in sequential schedule. The estimated 4-year OS, PFS, FFS, and EFS were 93%, 93%, 86%, and 69%, respectively.

Conclusions

Our national experience confirmed that patients treated with nilotinib-based regimens frontline obtain fast and high rates of complete cytogenetic and major molecular response. Of note, all progressions to AP/BP occurred in the first year, and all cases were fatal. Overall, 93% of the patients were estimated to be alive and progression-free at 4-years, with 69% of the patients still on nilotinib.

Acknowledgements

European LeukemiaNet, COFIN, Bologna University, BolognaAIL

Objectives and background

Treatment of cancers with cytotoxic agents often, but not always, results in transient to permanent testicular dysfunction. Imatinib mesylate (Glivec®, STI 571; Novartis), a small-molecular analog of ATP that potently inhibits the tyrosine kinase activities is one of the novel molecularly targeted agents being introduced into cancer therapy. This drug has demonstrated high level of efficacy in chronic myelogenous leukemia (CML), but it may interfere with Leydig cell activity and fertility. We aimed to determine cellular viability and apoptosis in mouse normal Leydig cell exposed to imatinib.

Methods

The mouse TM3 Leydig cells were cultured in DMEM-F12 medium supplemented with 10% fetal bovine serum, at 37°C in 5% CO2. On a 96-well plate, each condition was present in triplicate. Cells (3000 per well) were incubated in 100 µL of medium. Imatinib mesylate was prepared by a 10 mM stock solution in distilled sterile water and stored at –20°C. To determine the effect of imatinib, TM3 Leydig cells were treated with 0, 2.5, 5, 10 and 20 µM imatinib for 2, 4 and 6 days. The cellular viability and apoptosis levels were assessed by MTT and caspase-3 activities colorimetric methods, respectively. Statistical analysis was performed using the SPSS software. For data analysis, One-Way ANOVA and t-test were performed. A p-value <0.05 was considered for significant difference.

Results

In the treated and control cells, mean±SD of cellular viability (0.016±0.006 and 0.028±0.003) was statistically different (p<0.05) but apoptosis level had no significant difference between groups. With increasing drug dosage cellular viability decreased significantly (p<0.05) but apoptosis level did not show significant variation in different dosage of treatment. None of the results differed depending on exposure duration.

Conclusions

By decreasing cellular viability in short time after exposure, imatinib may reduce fertility potential in patients treated with this drug. The lack of apoptosis changes in the present study may be due to increased activity of different pathways leading to cell death such as necrosis-like programmed cell death. Another explanation may relate to the types of cell which was normal and not cancerous. These cells may be less affected by exposure to imatinib. This subject should be evaluated by comparing multiple cell death pathways between normal and cancer cells and also by complete and long term studies.


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[10] The effect of imatinib on mouse normal Leydig cell viability and apoptosis

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Background
Treatement of chronic myeloid leukemia (CML) with tyrosine kinase inhibitors (TKI) and its impressive results led to an increasing importance and urgency of the issues of pregnancy in patients dealing with CML during this period.

Methods
A total of 14 pregnancies, detected during chronic phase CML in the period of 2009-2013, were analyzed. 9 pregnancy cases involved 7 women with leukemia; other 5 pregnancies involved 4 partners of male patients treated for CML. Certain cases were consulted at FSBI Hematology Research Center, Russian Federation.

Results
Pregnancies in 7 women were further subdivided to distinguish 4 cases with imatinib therapy during pregnancy and a subgroup without treatment in this period (5 cases). Three patients from the first subgroup took IM at conception and during first 3-4 weeks of pregnancy stopping treatment immediately after becoming aware of their pregnancy. Before conception, one of these patients had minor cytogenetic response, the second one a complete cytogenetic response (CCyR), the third had major molecular response (MMR). The first of these patients resumed IM (400 mg/day) at 22nd-23rd week of pregnancy due to loss of complete hematological response. She subsequently gave birth to a healthy child and continued IM. Other 2 patients also delivered healthy children in due term while one of them (with MMR) maintained her response through all pregnancy and after delivery, whereas the other (with CCyR) lost her response by conclusion of pregnancy. The fourth patient of this group was diagnosed with CML at the 22nd week of pregnancy and took IM (400 mg/day) from the 26th week. Her pregnancy concluded with delivery of a healthy newborn. Patients of the second subgroup did not take TKI during their pregnancies. In two of them TKI was interrupted 2-3 months prior to conceiving, both of the patients were in CCyR. In one case, an early miscarriage occurred whereas the second patient had no complications and gave birth to a healthy child. She maintained CCyR until pregnancy conclusion without treatment and resumed IM after delivery. Other 2 women of this subgroup were diagnosed with CML at different time-points during pregnancy (<12 weeks and 32nd-33rd week) and delivered healthy infants. Both of them started IM after delivery. In the 5th case, pregnancy was terminated for medical reasons after diagnosing CML. In this study, we also analyzed 5 cases of pregnancies in partners of 4 male patients with CML. Treatment with IM was interrupted before conception in one case, all other pregnancies occurred during imatinib 400 mg/day. In all 5 cases pregnancies had no complications and concluded with deliveries of 5 healthy newborns.

Conclusions
Results of this small study suggest that management of CML in pregnancy should depend on the depth and duration of CML response and term of pregnancy. Taking into account the increasing number of pregnancies in CML patients and variations in their course and management, a need for a unified database becomes very urgent, in order to study bigger numbers of such cases and create clear recommendations for their management.

[12] Overcoming tyrosine kinase inhibitor (TKI) resistance with interferon-alfa (INF-α) in high-risk chronic myeloid leukemia (CML) patients

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Objectives and background
TKIs have become a golden standard in the current treatment of CML. TKI resistance is one of the major causes of treatment failure. INF-α was one of the main treatment options before the discovery of TKIs. Reports of complete cytogenetic responses (CCyR) and complete molecular responses (CMR) after INF-α associated with excellent long-term prognosis have been published. We report 3 high-risk CML patients treated with INF-α alone or in combination with TKI to successfully overcome TKI resistance.

Methods
Diagnosis, cytogenetic and molecular monitoring was performed according to ELN guidelines. Sanger sequencing was used for identification of BCR-ABL domain mutations. Roferon (Roche, Basel, Switzerland) was used for the treatment at 3 MU daily or three times per week.

Results
(1) A 44-year-old male high-risk CML patient was treated with imatinib for 34 months, achieving only a partial cytogenetic response. The patient was switched to dasatinib achieving a major molecular response (MMR), nevertheless the T315I mutation was detected after 8 months of therapy. Since there was no HLA identical donor available, treatment with INF-α was initiated. First CMR was achieved after 17 months of INF-α administration. Since then the patient has maintained fluctuating levels of BCR-ABL transcript between MMR and CMR and has continued INF-α for more than 60 months.
(2) A 59-year-old female high-risk CML patient was diagnosed in accelerated phase (AP). Conventional cytogenetics revealed additional cytogenetic abnormalities. After initial cytoreduction, imatinib was started at the maximum tolerated doses for 19 months and CCyR and MMR were achieved, however, a cytogenetic relapse occurred after 17 months and the M351T mutation was detected. The patient was switched to dasatinib therapy reaching CCyR and MMR. Nevertheless, after 22 months on dasatinib, BCR-ABL transcript levels started increasing and the M351T and F317L mutations were revealed. The therapy was changed to nilotinib, and after 3 months of nilotinib monotherapy, INF-α was added at minimal doses maintaining the patient in MMR for 12 months and achieving CMR after the 13th month of treatment.
(3) A 39-year-old male high-risk CML patient was diagnosed in the AP. There was an additional Philadelphia chromosome detected in one clone. The patient was treated with imatinib for 3 years. The treatment was complicated with non-compliance to the therapy, leading to dose alterations and interruptions. However, CCyR was achieved and maintained for 2 years. Then, cytogenetic relapse occurred and the patient was switched to dasatinib. However, the T315I mutation was detected after 5 months of treatment. Since the patient refused stem cell transplantation, INF-α was started and clearance of the mutated clone followed. At the same time, increased BCR-ABL transcript levels were observed and thus nilotinib was added to INF-α. A significant decline of BCR-ABL transcript levels was noted after two months of treatment.

Conclusions
Our clinical cases demonstrate the usefulness and safety of INF-α even in high-risk CML patients harboring TKI resistant mutations including T315I mutation when bone marrow transplantation cannot be performed. INF-α still represents a valid option for the treatment of individually selected CML patients in TKI era.

Support
Supported by the grant IGA UP LF-2013-004.
Background

Success in tyrosine kinase inhibitor (TKI) treatment of chronic myeloid leukemia (CML) is highly related to following of the established rules of monitoring and making effective therapeutic decisions in time. The evaluation of quality of routine monitoring in clinical practice is needed to understand the general picture of treatment results.

Aim

To analyze the quality of monitoring in clinical practice in CML patients (pts) treated in routine clinical practice in Russian Federation.

Methods

The analyzed cohort consisted of 201 pts from 6 regions of Russia with Ph/BCR-ABL-positive CML diagnosed between 01.10.2009 – 31.12.2012 (PBS EU-TOS). Median (Me) age was 50 (18-82) years (y), M:F% ratio 49:51%. Pretreatment: Hydroxyurea in 99 (52%) pts and IFNα in 2 (1%) pts. Me duration of CML prior to the TKI therapy was 0.65 (0-6.75) mo. Me period from CML treatment start was 1.5 (0-3.3) y. Chronic phase (CP), accelerated phase (AP), and blast crisis (BC) was diagnosed in 189 (94%), 11 (5.5%) and 1 (0.5%) pts respectively. Me duration of 1st-line TKI-therapy was 15.4 (0-38.9) mo (June 2013); 8 pts were without any TKIs (3 of them died; 3 of them diagnosed in Dec 2012; 2 of pts need to validate data).

Results

189 pts are treated with TKIs and now alive. Overall survival in the analyzed cohort is 85% (Log-rank <0.0001). 21 of 201 pts died (10.4%). The 1st line TKI therapy was imatinib (IM) and nilotinib (NIL) in 174 and 7pts, respectively; 2nd line TKI therapy was NIL for 10 pts and dasatinib (DAS) for 1 pt. To characterize the monitoring of IM therapy at mo. 164 pts were analyzed (25 pts were not included due to observation period ≤4 mo). Monitoring at 3 mo was not performed in 91 (56%) pts; cytogenetic study (CySt) + molecular study (MoSt) in 35 (21%) cases; only CySt and only MoSt in 21 (13%) and 17 (10%) cases, respectively. Results of CySt at 3 mo: 36 pts achieved major cytogenetic response (MCyR), 13 of them complete cytogenetic response (CCyR), 1pt minor CyR. Generally, 19 pts had >65% Ph-chromosome. Results of MoSt at 3 mo: the level of BCR-ABL expression ≤0.1-1%, 1-10% and >10% was achieved in 13, 19 and 20 pts respectively. At 6 mo of IM therapy, 149 pts were analyzed (40 pts had observation period ≤7 months). Lack of studies was in 68 (45%) pts, CySt+MoSt performed in 38 (25%) cases, only CySt and only MoSt in 29 (20%) and 14 (10%) pts respectively. Results of CySt at 6 mo: 50 pts achieved MCyR (33 of them CCyR), 7 pts minorCyR, 10 pts had >65% of Ph-chromosome. Results of MoSt at 6 mo: the level of BCR-ABL expression ≤0.1%-1%, 1-10% and >10% was achieved in 26, 17 pts and 10 pts respectively.

Conclusion

The lack of cytogenetic and molecular monitoring in routine clinical practice makes it difficult to evaluate therapy results in time. Monitoring should be improved in order to minimize treatment failure in CML patients.


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[14] Low expression of the beta-catenin antagonist CBY1 in chronic myeloid leukemia is driven by DNA hypermethylation

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Objectives and Background

BCR-ABL fusion gene is the causative genetic lesion of chronic myeloid leukemia (CML). However, it has a marginal role in leukemic stem cell (LSC) proliferation and survival, mostly contingent upon beta-catenin signalling.

Beta-catenin is a central component of BCR-ABL+ LSC self-renewal and persistence under tyrosine kinase (TK) inhibitor therapy. Multiple events concur to beta-catenin stabilization. Its nuclear import to form a transcription complex with T-cell factor (TCF) / lymphoid enhancer factor 1 (LEFI) activates the expression of target genes, such as MYC and cyclin D1. CBY1 is a beta-catenin antagonist which competes with beta-catenin for binding with TCF/LEFI and promotes beta-catenin–nuclear export, hence acting as a tumor suppressor. Our recent study supports that its BCR-ABL-associated reduction may be a component of activation of beta-catenin signaling in CML LSC.

DNA methylation is a key epigenetic modification for cellular processes and it predominately occurs at the CpG dinucleotides, where DNA methyltransferases (DNMT) mediate the transfer of methyl groups to cytosines hence generating 5-methylcytosines (5mC). The CpG island methylator phenotype is a common event in CML eventual-ly associated with the disease progression and drug resistance outcome.

Here we investigated the methylation pattern of CBY1 promoter in the LSC compartment and the role of DNMT1 in CBY1 promoter methylation status.

Methods

K562 treated with imatinib and 5-azacytidine and CD34+ cells from bone marrow samples of six CML-CP patients were compared with more differentiated hematopoietic progenitors contained in mononuclear cell fractions for CBY1 transcript level and for 5 methyl cytosine (5mC) content and DNMT1 presence at a CBY1 promoter region encompassing the region -85 to +120.

Results

Leukemic CD34+ cells displayed a transcription downmodulation of CBY1 driven by DNA hypermethylation at promot-e-associated CpG islands of CBY1-encoding gene. CBY1 promoter hypermethylation was at least partly contingent upon the enhanced recruitment of DNA methyltrans-

ferase (DNMT1) 1 at the aforesaid promoter region. The reduction of CBY1 expression drives an increment of cyclin D1 mRNA in the LSC compartment due to beta-catenin nuclear localization. The re-establishment of CBY1 expression in response to imatinib and 5-azacytidine drives beta-catenin cytoplasmic relocation.

Conclusion

Our study suggests that CBY1 expression was remarkably reduced in the putative BCR-ABL+ LSC compartment identified by a CD34+ phenotype compared to differenti-at-ed leukemic progenitors. CBY1 reduction was evoked by transcriptional downmodulation driven by the DNA hypermethylation and DNMT1 recruitment at promoter-associated CpG islands of CBY1-encoding gene. DNA hypermethylation has been involved in BCR-ABL-driven silencing of putative tumor suppressor genes, eventually associated with the disease progression and/or drug resistance. Its role in CBY1 downmodulation leading to beta-catenin activation may be critical in LSC survival and self-renewal. These findings suggest the putative advantage of de-methylating agents in association with TK inhibitors for CML therapy.
[15] Genomic BCR-ABL1 breakpoints in chronic myeloid leukemia characterized using long-range PCR and Next Generation Sequencing (NGS)

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The objective of our study was to detect genomic breakpoints in BCR-ABL1 using multiplex long-range PCR (LR-PCR) and Next Generation Sequencing (NGS) in forty-eight CML patients. We identified 11 SNPs and 32 INDELs in BCR and 22 INDELs in ABL1. The most frequent SNP was G/A (rs140506) found in 15/21 patients and K562. SNP G/A (rs140506) was found in 15/21 patients and K562. SNPs were detected in BCR; 11 SNPs (median 3 SNPs/patient, range 1-11) were found in 9/21 patients, none in K562. Sixty four SNPs were detected in BCR1; 11 SNPs (median 3 SNPs/patient, range 1-11) were included in this study. The K562 cell line with a characterized genomic BCR-ABL1 breakpoint (Krumholz et al 2012) was used as control. DNA was isolated from leukocytes of peripheral blood at diagnosis. We performed 2 rounds of LR-PCR. In the 1st round, 1 forward primer located in BCR and 10 primers located in ABL1 (Lange et al 1999) were used for multiplex LR-PCR. LR-PCR products were obtained in 28/49 cases. The 2nd round of multiplex LR-PCR was performed for the rest of samples using 3 BCR and 20 ABL1 primers (Krumholz et al 2012, Ross et al 2010, Score et al 2010). LR-PCR products were prepared for NGS including nebulization to short fragments and rapid library preparation for Junior platform (Roche AppliedScience). NextGENe software (Softgenetics) was used for sequence analysis and breakpoints characterization. NCBI database and reference sequences for BCR and ABL1 genes were used for breakpoint location and sequence features identification. Sanger sequencing is not suitable for sequencing of long PCR products, thus another set of experiments was sought for BCR-ABL1 fusion identification. The multiplex LR-PCR followed by NGS accelerated the whole process. Moreover, this approach enables to analyse long sequences downstream and upstream from the BCR-ABL1 fusion and to identify other sequence features.

[16] Cytoplasmic compartmentalization of beta-catenin following Chibby enforced expression activates autophagy as pro-survival mechanism in cells expressing the BCR-ABL1 fusion gene

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Autophagy is a genetically regulated process of adaptation to metabolic stress. Besides its controversial role in tumor cell survival, it may promote tumor cell adaptation to pharmacological treatment through events encompassing the metabolic reprogramming. The participation of autophagy in CML cell death in response to tyrosine kinase inhibitors supports that autophagy may be a component of BCR-ABL1 leukemogenic potential. Here we report that beta-catenin nuclear export and inactivation driven by the enforced expression of its antagonist Chibby (CBY) activates autophagy in K562 cell line. Beta-catenin translocation from nuclear into cytoplasmatic compartment was associated with a significant increase of different markers of autophagy such as Beclin 1, the core protein of autophagy activation process, enhanced conversion of cytosolic-associated protein light chain 3, a critical step for autophagosome maturation, and presence of autophagosomes, detected by immunofluorescence microscopy.

Methods

Forty eight CML patients who responded to treatment with deep molecular response were included in this study. The K562 cell line with a characterized genomic BCR-ABL1 breakpoint (Krumholz et al 2012) was used as control. DNA was isolated from leukocytes of peripheral blood at diagnosis. We performed 2 rounds of LR-PCR. In the 1st round, 1 forward primer located in BCR and 10 primers located in ABL1 (Lange et al 1999) were used for multiplex LR-PCR. LR-PCR products were obtained in 28/49 cases. The 2nd round of multiplex LR-PCR was performed for the rest of samples using 3 BCR and 20 ABL1 primers (Krumholz et al 2012, Ross et al 2010, Score et al 2010). LR-PCR products were prepared for NGS including nebulization to short fragments and rapid library preparation for Junior platform (Roche AppliedScience). NextGENe software (Softgenetics) was used for sequence analysis and breakpoints characterization. NCBI database and reference sequences for BCR and ABL1 genes were used for breakpoint location and sequence features identification.

Results

Median length of LR-PCR products was 6 kb (range 1-10kb). NGS has been performed in 22/49 samples so far with coverage 50-20000 reads per base. Breakpoints characterization was confirmed by Sanger sequencing. Eighteen BCR breakpoints (82%) were located in intron 14 and 4 (18%) in intron 13. Except of 1/22 sample in which the ABL1 breakpoint was located on exon 1a, all breakpoints were dispersed across intron 1. Altogether 73 annotated SNPs in BCR gene were detected in 15/21 patients (median 3 SNPs/patient, range 1-12) and K562. Thirteen SNPs were found in at least 2 patients. SNP G/A (rs140506) was found in 15/21 patients and K562. INDEL-IC (rs66503844) was identified in 6 patients. Forty six SNPs were detected in BCR1; 11 SNPs (median 3 SNPs/patient, range 1-11) were found in 9/21 patients, none in K562. Each of 10 SNPs was identified in 2 patients. In one patient INDEL-IA (rs35152617) was identified. Analysis of repeat elements and motifs associated to DNA recombination are ongoing.

Conclusions

Sanger sequencing is not suitable for sequencing of long PCR products, thus another set of experiments was sought for BCR-ABL1 fusion identification. The multiplex LR-PCR followed by NGS accelerated the whole process. Moreover, this approach enables to analyse long sequences downstream and upstream from the BCR-ABL1 fusion and to identify other sequence features.

Supported

IGA/NT11555.
CML

Objectives and background
Most patients with CML in chronic-phase (CML-CP) treated with imatinib have shown an overall survival rate of 85% in the 8 year update of IRIS trial, but only a minority of imatinib-treated patients achieve complete molecular responses (CMR). Although second-generation tyrosine kinase inhibitors (TKIs) yield higher rates of CMR versus imatinib, there is still no evidence to support the eradication of CML stem cells. Recent evidence suggests that upon TKI treatment, CML stem cell survival is BCR-ABL kinase independent. The bone marrow (BM) is a dynamic microenvironment with a high concentration of soluble factors that regulate hematopoiesis, enhance leukemia blast survival and modulate their resistance to pharmacological treatment.

Results
Ph+ K562 cells were cultured in RPMI medium, defined as regular medium (RM). The human stroma cell line HS-5 serum-free supernatant was used as feeder (HS5/SCM). The apoptosis of Ph+ K562 cell line, treated with clinical doses of imatinib, nilotinib or dasatinib on HS-5 monolayer is significantly reduced (18%±13%, 50%±6%, or 10%±10%, respectively), with respect to RM (46%±12%, 84%±15%, or 53%±20%, respectively). Moreover, the TKI-resistance is also related to soluble factors produced by HS-5 cells. Indeed, apoptosis is greatly reduced when the K562 cell line is treated with imatinib, nilotinib or dasatinib in the presence of HS-5/SCM (20%±9%, 29%±18%, or 17%±6%, respectively), with respect to RM. Furthermore, the IC50 of imatinib, nilotinib or dasatinib is significantly increased when K562 cells are cultured on HS-5/SCM (7957nM, 889nM, 2.5nM, respectively) vs on RM (545nM, 13.93nM and 1.12nM, respectively). Upon SCM exposure, Ph+ cells, treated with TKIs, preserve long-term ability to re-start proliferation in vitro after TKI withdrawal.

Conclusions
Indeed, the resistance to TKI treatment in this stromal co-culture experimental model is associated to BCR-ABL-independent STAT-3 activation. Furthermore, we demonstrated that the JAK inhibitor ruxolitinib synergizes with TKIs to induce apoptosis in progenitor CML cells and to reduce their clonogenic potential. Importantly, imatinib, nilotinib, and ruxolitinib, alone or in combination, did not significantly impair the formation of normal erythroid and myeloid colonies. Taken together, our data provide a rationale for the therapeutic combination of TKIs and ruxolitinib with the aim of eradication of primary BCR-ABL+ cells homed in BM niches.

Key words: chronic myeloid leukemia, accelerated phase, BCR/ABL, nilotinib, side-effects.

[17] Extensive Pleural and Peritoneal Effusion in Accelerated Phase of Chronic Myeloid Leukemia
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Nilotinib is considered an effective drug in imatinib-resistant chronic myeloid leukemia. Although reported to be well-tolerated, severe events such as pleural or peritoneal effusion have been reported at 800 mg daily.

We examined our chronic myeloid leukemia patient treated with nilotinib at 600 mg daily and identified this patient who developed marked effusion formation after 7 days of therapy, with grade III/IV pleural and/or peritoneal effusions recorded. This patient had received previous anti-leukemia therapy with imatinib and interferone but did not have preexisting peritoneal or pulmonary disease. In this patient, nilotinib was discontinued despite treatment with diuretics and glucocorticosteroids.

In conclusion, nilotinib-treated chronic myeloid leukemia patients are at risk for the development of pleural and peritoneal effusions even when the drug is administered at 600 mg daily. Therefore, all patients should be examined for pre-existing comorbidity and risk factors before starting nilotinib, and all should have repeated chest X-rays during long-term nilotinib therapy.

Key words: chronic myeloid leukemia, accelerated phase, BCR/ABL, nilotinib, side-effects.

[18] Ruxolitinib synergizes with tyrosine kinase inhibitors to overcome drug resistance related to bone marrow stroma microenvironment in chronic myeloid leukemia (CML)
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Extensive Pleural and Peritoneal Effusion in Accelerated Phase of Chronic Myeloid Leukemia
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Nilotinib is considered an effective drug in imatinib-resistant chronic myeloid leukemia. Although reported to be well-tolerated, severe events such as pleural or peritoneal effusion have been reported at 800 mg daily.

We examined our chronic myeloid leukemia patient treated with nilotinib at 600 mg daily and identified this patient who developed marked effusion formation after 7 days of therapy, with grade III/IV pleural and/or peritoneal effusions recorded. This patient had received previous anti-leukemia therapy with imatinib and interferone but did not have preexisting peritoneal or pulmonary disease. In this patient, nilotinib was discontinued despite treatment with diuretics and glucocorticosteroids.

In conclusion, nilotinib-treated chronic myeloid leukemia patients are at risk for the development of pleural and peritoneal effusions even when the drug is administered at 600 mg daily. Therefore, all patients should be examined for pre-existing comorbidity and risk factors before starting nilotinib, and all should have repeated chest X-rays during long-term nilotinib therapy.

Key words: chronic myeloid leukemia, accelerated phase, BCR/ABL, nilotinib, side-effects.
Objectives and background

Revolutionary improvement occurred in CML treatment in the last decade. At present, there is a gradual, sustained increase in the number of patients that leads to an avalanche-like increment of the budget burden. In the treatment of newly diagnosed CML patients, second-generation tyrosine kinase inhibitors (TKI) showed encouraging results with reduced frequencies of adverse events (AE) and substantial improvements of the rate and promptness of deep molecular responses in comparison with imatinib. Patients with complete molecular responses (CMR) are now considered as candidates for therapy cessation in clinical trials. Cessation trials revealed that 39 to 67% of enrolled patients can remain in sustained molecular response without TKI therapy for a long period of time. The aim of our study was to compare the costs of first- and second-generation TKI in first-line CML treatment.

Methods

We have used the Markov chain approach to compare CML first-line treatment strategies with imatinib or nilotinib with subsequent therapy cessation in cases of CMR achievement. The input parameters for transition rates were selected from clinical trials (IRIS, ENESTnd, DASISION, ENACT, CA180013, STIM, FILMC group), own data and experts’ opinions. We have chosen the model population size as 800 newly diagnosed CML patients in Russia annually. 20-years’ time horizon was used. We tried to assess the treatment worth for one patient and the cumulative budget burden for the whole national population of CML patients. The total cost included direct costs of diagnostic procedures to establish diagnosis, residual disease monitoring, costs of medications (TKI and concomitant drugs for AE management), and allogeneic stem cell transplantation. We have chosen the discount rate as 3% per annum. For representation of our results we recalculated total cost in Euros. Statistical methods included a simulation model.

Results

The results of our analysis at individual level showed that the average total cost for one patient in case of the use of nilotinib instead of imatinib in 1st line CML treatment is more expensive during the first three years. Subsequently, the 1st line nilotinib treatment strategy becomes cheaper due to more frequent successful therapy cessation. On the whole national level, we got similar results: The total CML budget burden in case of nilotinib 1st line treatment strategy was higher than imatinib 1st line during first ten years, but after that, more frequent therapy cessation results in cost saving (Fig.1). Importantly, the use of nilotinib in 1st line could save more than 500 CML patients’ lives from death without any additional expenses. We should note that the results of our analysis are strongly depended on input parameter values, which could be changed in future.

Conclusions

Pharmacoeconomic modelling can simulate budget burden and its future dynamics on the national level. The results of such modelling can be used in the decision making process for the national treatment standards development.

Figure 1. Nilotinib and Imatinib in first-line CML treatment on whole Russia budget burden.
Objectives and background
The generic substitution of original drugs is actively proposed by the WHO in order to reduce costs and improve access to effective drugs for more patients. In Russia, as in most countries, for the registration of generics, official regulations only require evidence of pharmaceutical and biological equivalence, but no evidence of efficacy or safety identity (therapeutic equivalence) is necessary. There are no requirements to similarities of synthesis processes, the quantity and species of by-products, fillers, and dosage forms. As a rule, patients have negative expectations about generic substitution – the “nocebo” effect. Since August 2012, original imatinib was almost totally replaced with two generic forms in Russia. The aim of the study was to assess the tolerability and efficacy of these generics in our centre.

Methods
79 CML patients initially treated with original imatinib (Novartis AG) with a median duration of treatment of 6.5 years (0.5-11 years) were switched to generics (switched patients, SWPTS), and 11 newly diagnosed CML patients (NDCML) were started with generics. Drugs: (1) GenericPh 100 mg in capsules (Ph-Syntez, Russia); (2) GenericG 100 mg in tablets (Laboratorio TUTEUR S.A.C.I.F.I.A., Argentina). Switching from one generic to another was made in cases of intolerance. We analysed the range and frequency of adverse events (AE), rates of complete hematologic (CHR), partial cytogenetic (PCyR) and complete cytogenetic (CCyR), and major molecular (MMR) responses in NDCML and its durability in SWPTS. As comparator for AE frequency on long-term treatment, we used IRIS data. The statistical analysis included Chi-square and Fisher exact tests. Here, we present the results of the 9-months cut-off analysis. In the meeting, the 1 year cut-off results will be presented.

Results
All 11 NDCML were treated with GenericPh for >3 months: 9/11 reached CHR (82%), 3/11 (27%) – PCyR, and 3/11 (27%) – CCyR (optimal response – 55%). 5/11 NDPTS had treatment duration >6 months: 3/5 achieved CCyR including 2 MMR (optimal response – 60%). 1/11 NDCML progressed to blastic phase, one patient was switched to nilotinib due to imatinib intolerance (oedema), one patient was switched to dasatinib due to the E255K mutation. 54/79 of SWPTS were treated with GenericPh and 25/79 SWPTS - with GenericG. Durability of pre-existing responses in SWPTS was as follows: 4 patients lost their MMR (3/54 [5.6%] on GenericPh and 1/25 [4%] on GenericG); 3 patients lost CCyR (2/54 [3.7%] on GenericPh and 1/25 [4%] on GenericG). Tolerability: 3/25 patients in the GenericG group had severe gastroenterological toxicity (nausea, vomiting, abdominal distension, diarrhoea) and were switched to GenericPh. This may be caused by the tablet filler (lactose) and related to lactase insufficiency. The frequencies of other AEs are presented in the table below. No significant differences were revealed between generics and original imatinib (according to IRIS data).

Conclusions
Our limited data did not reveal any significant differences in terms of efficacy and tolerability with the exception of the possible role of lactase insufficiency for GenericPh-treated patients. Our findings suggest the need of therapeutic equivalency evaluation in generic registration process to diminish alertness of physicians and their patients.

References
[21] Allogeneic hematopoietic stem cell transplantation (allo-HSCT) for chronic myeloid leukemia (CML): The Tunisian experience

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Objectives and background

In Tunisia, allo-HSCT for CML has been replaced by imatinib as front line therapy since 2003. The indication for transplantation was limited for patients with accelerated phase (AP), greater than chronic phase (CP>1), blast crisis (BC) and treatment failure (TF). Here, we compare our results before and during the imatinib era.

Patients and methods

Between June 1998 and March 2013, 34 patients were transplanted for CML. Before 2003, 19 patients (group1) with sibling donor and different disease phase (6 CP1, 11 AP or CP2, 2 BC) received allogeneic bone marrow transplantation. The median age was 24 years (range; 8-34). The median time from diagnosis to transplant was 6 months (range; 2-19). The EBMT score was mainly low of 0-2 (n=15, 78%). The median number of CMN was 2.5x10^8/kg (range; 1.15-3.87). Myeloablative conditioning regimens (Bu-Cy, TBI-Cy or Bu-Cy-VP16) were used. Since 2003, 15 patients (group 2) were transplanted after a first-line therapy by tyrosine kinase inhibitors (imatinib (n=11) and/or 2nd TKI (n=4)). Indications for transplantation were AP ± TF (n=7), BC (n=7) and CP1. The median time from diagnosis to transplant was 21 months (range; 5-30). The EBMT score was mainly high of 3-4 (n=11, 73%). Only 5 patients achieved major (MMR) or complete molecular response (CMR) with TKIs. Conditioning regimen are Bu(iv)-Cy and TBI-VP16. Peripheral blood stem cells was the main source of SC (n=1) with a median number CD34+ cells of 4x10^6/kg (range; 1.8-7.62). GVHD prophylaxis associated cyclosporine A and short course of methotrexate.

Results

Group 1: Mortality was mainly due to transplant-related toxicity (n=9, TRM=47%) or hematologic relapse (2 patients in BC, 10.5%). Three patients with cytogenetic relapse (30%) received escalating doses of donor lymphocyte infusions (DLI) and could restore durable complete molecular remission (CMR). After a median follow-up of 42 months (range; 1-178), 8 patients are alive with CMR. The overall survival rate is of 43% at 3 years.

Group 2: TRM was significantly lower (n=3; 20%). One patient died from relapse with BC (6%). Two patients with molecular relapse (16%) received imatinib and could restore CMR. After a median follow-up of 38 months (range; 3-87 months), 11 patients (73%) are alive with CMR. Only 1 patient has an extensive chronic GVHD. The overall survival rate is 74% at 3 years and significantly higher than of the group1 (p=.001).

Conclusion

Transplantation for CML patients is associated with high mortality. First-line therapy by TKIs, even in the advanced phases and high EBMT score, significantly improved results. Pre-emptive use of TKI after transplantation could restore CMR without risk of cGVHD.

[22] A novel t(3;12)(q21;p13) translocation in a patient with accelerated chronic myeloid leukemia after imatinib and nilotinib therapy

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Objectives and background

The acquisition of secondary chromosomal aberrations in chronic myeloid leukemia (CML) patients with Philadelphia chromosome-positive (Ph+) karyotype signifies clonal evolution associated with the progression of the disease to its accelerated or blast phase. Therefore, these aberrations have clinical and biological significance. t(3;12)(q26;p13), which is a recurrent chromosomal aberration observed in myeloid malignancies, is typically associated with dysplasia of megakaryocytes, multi-lineage involvement, short duration of any blast phase, and extremely poor prognosis.

Methods

We have identified a recurrent reciprocal translocation between chromosomes 3 and 12 with different breakpoint at bands 3q21 and 12p13 in the malignant cells from a 28-year-old man. The patient was initially diagnosed as having Ph+ CML in the chronic phase. The t(3;12)(q21;p13) translocation occurred 4 years after the patient was first diagnosed with CML while undergoing tyrosine kinase inhibitor therapy. We confirmed the t(3;12)(q21;p13) translocation via fluorescence-in-situ-hybridization by using whole-chromosome paint probes for chromosomes 3 and 12.

Results

Our findings demonstrate that, similar to other recurrent translocations involving 3q26 such as t(3;3) and t(3;21), the t(3;12)(q21;p13) translocation is implicated not only in myelodysplastic syndrome and acute myeloid leukemia but also in the progression of CML.

Conclusions

These findings extend the disease spectrum of this cytogenetic aberration.
[23] Usefulness of vertebral and pelvis bone marrow MRI assessment on patients with primary or secondary myelofibrosis and correlation with grade of fibrosis

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Objectives and background
Polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF) are Philadelphia-negative chronic myeloproliferative neoplasms that share clinical and histopathologic features as the ominous transformation of PV or ET to MF. The histological exam of bone marrow (BM) by biopsy in iliac crest is an aggressive test mandatory for the diagnosis and follow-up of MF; providing the evidence of increase of reticular or collagen fibers. However, the usefulness of MRI for bone marrow assessment have been proved in Gaucher Disease (Med Clin 2011; 137(12):3-31) and in hematological malignancies as acute leukemias and multiple myeloma. Here we present preliminary results derived of the assessment of BM by MRI on patients with MF.

Aims
To correlate the fibrotic distribution pattern of bone marrow in iliac crest biopsy in patients with MF with the results in bone marrow distribution in spine and iliac bone, to define the distribution pattern of bone marrow by MRI in patients with MF and correlate with histologically defined fibrotic changes.

Methods
A clinical institutional protocol was performed including 8 patients with primary or secondary to Ph(-) MPN BM MF grade II-IV diagnosed by bone marrow biopsy. MRI of spine and pelvis were performed for each one (same machine and same radiologist) and compared with a control group of 8 non-hematological patients (control group).

The radiologist should define BM radiological pattern in a blinded manner (no information about grade of MF were provided). A database was performed with clinical, histological and MRI data for analysis.

Results
Of 8 patients, M/F ratio was 4/4, mean age at diagnosis: 66.3 y.o. (53-75). Diagnostics: primary MF: 2, MF secondary to PV: 2 and to TE: 4 patients. DIPSS prognostic score: low: 2, intermediate-2: 4, high: 2. Histologic BM MF. Grade II: 3, Grade III: 4, Grade IV: 1. According to control group and previous radiologist experience on BM MRI assessment, the defined patterns of BM from less to intense infiltration were normal according age (NP), hematopoietic hyperplasia (HP), reticular infiltration (RI), speckle infiltration (SI), diffuse heterogeneous infiltration (DI) and diffuse homogeneous infiltration (HI).

Correlation between BM biopsy and MRI shows: for Grade II MF: HP: 1 patient, RI: 1 patient and SI: 1 patient, Grade III: SI: 2 patients and DI: 2 patients, Grade IV: HI: 1 patient.

Conclusions
These preliminary data confirm the usefulness of MRI on BM-MF assessment for MF patients. A full description of MRI patterns, correlation of MRI pattern and histology images and a quantitative analysis of pixels of a region of interest area using eFilm software (Merge HelthcareTM 3.0) of MRI will be presented.

[24] Follow-up of concurrent Jak2V617F mutation and BCR-ABL translocation in two cases of myeloproliferative disorders during therapy

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Objectives and background
In rare cases of concurrent JAK2V617F mutation and BCR-ABL translocation, the existence of two separate diseases or one malignant clone with both lesions is still unknown. We performed a follow-up of both molecular markers in two patients with co-existent JAK2V617F and BCR-ABL p210 during therapy and studied the cell fraction involvement into JAK2V617F genetic lesion in one case.

Methods
JAK2V617F was detected by real-time allele specific polymerase chain reaction (PCR) (sensitivity 0.01%) with DNA cell line UKE1 containing 100% of JAK2V617F as a positive control. JAK2V617F calculated towards housekeeping control gene ABL. BCR-ABL/ABL detected by real-time PCR, kits by Interlabservice (Moscow). Cell fractions (CD34+, CD8+, CD19+, CD15+) were extracted from 3 ml bone marrow (BM) aspirate by immune magnet separation (Miltenyi Biotec). Molecular evaluation was performed at diagnosis and during treatment.
Results
Clinical case 1. A 56 year old woman was diagnosed with primary myelofibrosis (PMF) in 2007. Multiple stroma fibrosis and increased mature granulocytopenia was observed in BM; splenomegaly 226 x 87 mm by ultrasound examination; red blood cells (RBC) 7.42 x 10^{12}/L, white blood cells (WBC) 14.90 x 10^{9}/L in peripheral blood (PB); positive for JAK2V617F. After 5 years of hydroxyurea treatment, WBC increased to 30.5 x 10^{9}/L, immature granulocytes and myelofibrosis increased in BM. BCR-ABL p210 transcript was detected (49.53%).

Chronic myeloid leukemia (CML), chronic phase, was established. BCR-ABL tyrosine kinase inhibitors (TKI) treatment was started: Imatinib 400 mg daily for 2.5 months, but switched to nilotinib 800 mg daily for the next 10 months due to intolerance. PB analyses became normal, but splenomegaly persisted, hydroxyurea was re-administered. BCR-ABL transcript decreased to 1% with no change of the JAK2V617F mutated clone level.

Clinical case 2. A 60 year old man was diagnosed with CML, chronic phase, in October 2012 with the typical picture of WBC 144 x 10^{9}/L, platelets 904 x 10^{9}/L, myelocytes 13%, metamyelocytes 1%, bands 32%, neutrophiles 36%, eosinophiles 8%, basophiles 2%, lymphocytes 3%: hematogamy + 3cm, splenomegaly +15cm, Ph-chromosome positive. Coexistence of 80% BCR-ABL p210 and 73% JAK2V617F was detected.

3 months after imatinib 400 mg daily therapy, the PB values and karyotype were normal, thrombocytosis and splenomegaly persisted. A trepanobiopsy proved the diagnosis of PMF. After 8 months of imatinib treatment, the BCR-ABL levels decreased to 0.081% (major molecular response). Interestingly, the mutated JAK2V617F clone decreased from 73% to 21%.

JAK2V617F quantitative analysis in BM cell fractions demonstrated the following level of the JAK2V617F clone: 90.13%, 69.02%, 57.83% and 26.98% in CD34+, CD19+, CD15+ and CD8+ cells consequently.

Discussion and conclusions
JAK2V617F coexistence with BCR-ABL has no influence on the effectiveness of TKI therapy although the clinical abnormalities (trombocytosis, splenomegaly) persist obviously due to the JAK2V617F clone. Different kinetics of the BCR-ABL-positive and Jak2V617F-positive clones during TKI therapy probably demonstrate bi-clonal leukaemogenesis. JAK2V617F is represented in lymphoid and myeloid BM cell fractions not equally. The primacy of either BRC-ABL or JAK2 lesions is discussable and needs further investigation. The opportunity for additional therapeutic interventions (interferon, JAK2 inhibitors) is also opened.

[25] The combination of panobinostat and ruxolitinib exerts synergistic effects to overcome bone marrow stroma protection in Philadelphia negative myeloproliferative neoplastic cells

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Objectives and background
JAK2V617F constitutively deregulates signaling of the JAK/STAT pathway conferring proliferative and survival advantages in chronic Ph-negative myeloproliferative neoplasms (MPNs). Many drugs target different pathways critical for MPN development, like the JAK inhibitor ruxolitinib, known to decrease spleen size and alleviate constitutional symptoms in myelofibrosis (MF). Other drugs work through remodeling of chromatin with a key role in epigenetics, like the pan-histone de-acetylase inhibitor panobinostat. In a phase I study for patients with MF, this drug showed to be clinically active, regardless of the JAK2 V617F status. Indeed, several mechanisms of resistance have been described. In this scenario, it is well known that BM stromal cell components create a favorable pathologic microenvironment in myelofibrosis that nurtures and protects the malignant cells. In this regard, we prove that the cytotoxic activity of either ruxolitinib or panobinostat is significantly inhibited by bone marrow (BM) stromal soluble factors, and that the combination of the two inhibitors synergizes to overcome the observed BM stroma-related resistance.

Methods
JAK2V617F tumor cell lines HEL and SET2 were treated with ruxolitinib and panobinostat in RPMI medium, defined as regular medium (RM), or on monolayers of stroma cell line HS-5 or bone marrow stroma secreted cytokines, defined as HS5/SCM. Isolated BM progenitors MPN cells were treated with both drugs in RM or HS5/SCM.

Results
In RM condition, panobinostat or ruxolitinib induced a significant apoptosis in SET2 and HEL cells in a dose-dependent manner. Indeed, when SET2 cells were treated with 30nM panobinostat or 300nM ruxolitinib in the presence of HS-5/SCM, the drug-related apoptosis is significantly reduced (40% ±18% and 30%±8%, respectively) with respect to cell line treated in RM (79%±15% and 58%±12%, respectively; p<0.05). Simultaneous results have been achieved for the HEL cell line but only when cells were treated with panobinostat (22%±4% in HS-5/SCM vs 46%±6% in RM; p<0.05), since ruxolitinib exerts no effect on HEL viability. The IC50 of SET2 cells treated with panobinostat or ruxolitinib is significantly increased in the presence of HS-5/SCM (31nM and 1222nM, respectively) versus the IC50 in RM (11nM and 305nM, respectively). Co-treatment of panobinostat and ruxolitinib strongly synergizes, increasing SET2 (96%±1%) and HEL (73%±5%) apoptosis, regardless HS5/SCM exposition. Importantly, we observed that whether the JAK inhibitor ruxolitinib or panobinostat are able to significantly reduce viability of CD34+ cells from MPN patients, independently of the JAK2V617F mutation. Moreover, although HS5/SCM exposition protects MPN primary progenitor cells from the cytotoxic activity of both drugs, we demonstrated that their combination is significantly synergistic to overcome BM stroma-related resistance. Finally, although co-treatment of MPN-CD34+ cells with panobinostat and ruxolitinib is not synergic to reducing CFU outgrowth with respect to the single agent panobinostat, it is significantly effective to reduce the count of MPNs CD34+ cells in long term sub-cultures.

Conclusions
Our data strongly support the hypothesis that a novel drug combination of ruxolitinib and panobinostat may be more effective in eradicating MPN cells curred in the BM rather than the single agents, and may significantly improve responses for patients with refractory disease and decrease the rate of therapy resistance.
[26] Detection of leukemia-associated mutations in haematologically normal elderly individuals

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Objectives and background
New sequencing technologies are facilitating the development of large gene panels that can be screened in patients with suspected haematological malignancies. Emerging evidence, however, has indicated that some malignancy-associated mutations might be detectable in the population at large, raising questions about the utility of broad mutation screening as a diagnostic tool.

Methods
We studied (i) the Uppsala Longitudinal Study of Adult Men (ULSAM) cohort, an ongoing epidemiological study of all available men born between 1920-24 in Uppsala County, Sweden, and (ii) 56 normal elderly women with known X-chromosome inactivation patterns (XCIIP) in both T-cells and neutrophils.

Results
In a pilot SNP array study of 108 ULSAM cases using the Illumina 1M-Duo beadchip analysis, one haematologically normal individual was identified who had acquired a large (103Mb) region of terminal acquired uniparental disomy (aUPD) at chr 4q (Forsberg et al., AJHG. 2012;90:217-28). Since 4q aUPD in myeloid malignancies is associated with TET2 mutations, we sequenced this gene and identified a 21bp deletion that removed part of exon 4 and is therefore likely to be deleterious. The TET2 mutation was absent from B-cell, T-cell and fibroblast DNA but present in leukocyte and granulocyte DNA extracted at the age of 90 as well as leukocyte DNA at ages 71, 82 and 88. To determine if aUPD is more widespread in elderly men, we analysed array data from a further ~1100 ULSAM cases and identified 14 individuals (1.3%) with aUPD (median size = 27Mb; range 13-87) at 1p, 6p, 9p, 9q, 11p, 11q, 14q, 15q, 17p, 19q and 22q. Of these, only two had been diagnosed with a haematological malignancy: one with JAK2 V617F positive PV (9p) and another with CLL (17p; p53 not tested). We did not detect MFL or CBL mutations in the cases with 1p or 11q aUPD; candidate somatically mutated genes have not been identified for the other chromosome arms. We then analysed a cohort of 56 haematologically normal elderly women; 10 had skewed XCIIP in neutrophils but not T-cells, 21 had skewed neutrophils and skewed T-cells, 21 were balanced in both fractions and 4 were skewed in T-cells but not neutrophils. The skewing may simply be a consequence of age-related stochastic processes but might be caused by subclinical clonal expansion. We screened neutrophil DNA for mutations in JAK2 V617F, DNMT3A (exons 15-23) and TET2 (all coding exons). JAK2 V617F was not seen in any case but 2 variants in DNMT3A (G699D and R882C) and 4 in TET2 (R126H, G1365R, 91477insA_91478_91481delAGGT, 176230delA) were identified in 3 cases that were either absent or markedly reduced in T-cells from the same patient. Of note, DNMT3A R882C is seen recurrently in patients with leukaemia, and two of the TET2 mutations are frame shifts. Three TET2 missense variants (M1028I, M1701I and Y867H) were identified that were also seen in T-cells.

Conclusions
Overall, these findings reveal a complex pattern of mutations in elderly individuals and support the notion that somatically acquired driver variants are detectable in some individuals with otherwise apparently normal haematopoiesis.

[27] Genetic markers in diagnostics and prognosis definition of BCR-ABL-negative myeloproliferative neoplasms

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Objectives and background
Genetic mutations result in abnormalities of myelopoietic proteins and lie at the basis of myeloproliferative neoplasms (MPNs) development and its subsequent progression. Besides the well-studied prognostic molecular marker JAK2 (mutation V617F and mutations in exon 12) for BCR-ABL negative MPNs, some information exists about the significant role of epigenetic factors. According to the published data, mutations in the gene EZH2 (2q35), which encodes a histone methyltransferase, are found in 6% cases of primary myelofibrosis (PMF), 1% cases of polycythemia vera (PV) and 1.3% cases of essential thrombocytemia (ET). EZH2 mutations may be of prognostic value in MPN’s at the time of transformation to the blastic phase. The clonal hematopoiesis of BCR-ABL-negative MPNs is a matter of investigation. The goal of our research was to determine the frequency of mutations in EZH2 in two groups of patients with different chromosomal aberrations.

Methods
We examined 42 patients with BCR-ABL negative MPNs. The average age of patients was 58 years (range, 19 - 76). The first group included 17 patients with normal karyotype, and 14 patients with the isolated chromosomal aberrations del(13)(q22), del(20)(q12), Y that are associated with favorable prognosis, and add(22)(q13), del(1)(p32), del(6)(q15), t(10;12)(q22;p13) that are referred to as intermediate risk. In one patient (3.2%) with del(6)(q15), karyotype transformation occurred from PMF to MDS. The second group included 11 patients with complex abnormalities and the chromosomal aberrations +8, +7, inv(7)(p11q21) that are associated with unfavorable prognosis. Among them, 2 patients (18.2%) had transformation from PMF to MDS and AML. Polymorphism of JAK2 (V617F) was defined by PCR-RFLP assay. Mutations in exon 12 of JAK2, 8, 10, 17, 18 and 19 exons of EZH2 were defined by sequence analysis.

Results
The frequency of mutations in JAK2 was 67.7% (21/31 cases) for V617F mutation and 6.4% (2/31 cases) for exon 12 in the favorable-intermediate group. The frequency of V617F mutation in JAK2 was 54.5% (6/11 cases) in unfavorable prognosis group. The ile713Thr mutation in EZH2 gene was detected in 2.4% (1/42 cases). The patient with this EZH2 gene mutation had a del(6)(q15) karyotype which is associated with intermediate risk, and he subsequently underwent transformation from PMF to MDS in 9 months after the disease onset.

Conclusions
Integration of cytogenetic and molecular analyses could be a valuable option for stratification of patients and optimising the treatment strategy. Mutations in the EZH2 gene could be assessed as additional prognostic markers of unfavourable prognosis in patients with BCR-ABL negative MPNs with different chromosomal aberrations.
[28] PVSG, and WHO versus European Clinical, Molecular and Pathological (WHO-ECMP) criteria for prefibrotic myeloproliferative neoplasms

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Abstract
The PVSG, WHO and European Clinical, Molecular and Pathological (ECMP) classifications agree upon the diagnostic criteria for polycythemia vera (PV) and advanced primary myelofibrosis (PMF). Essential thrombocythemia (ET) according to PVSG and 2007/2008 WHO criteria comprises three variants of JAK2V617F mutated ET when the ECMP criteria are applied. These include normocellular ET, hypercellular ET with features of early PV (prodomal PV), and hypercellular ET due to megakaryocytic, granulocytic myeloproliferation (ET.MGM). Evolution of prodomal PV into overt PV is common. Development of myelofibrosis is rare in normocellular ET (WHO-ET) but rather common in hypercellular ET.MGM. The JAK2V617F mutation burden in heterozygous mutated normocellular ET and in heterozygous/homozygous or homozygous mutated PV and ET.MGM is of major prognostic significance. JAK2/MPL wild type ET associated with prefibrotic primary megakaryocytic and granulocytic myeloproliferation (PMGM) is characterized by dense clustered immature dysmorphic megakaryocytes with bulky (bulbous) hyperchromatic nuclei, which are never seen in JAK2V617F mutated ET, and PV and also not in MPL515 mutated normocellular ET (WHO-ET). JAK2V617F mutation burden, spleen size, LDH, circulating CD34+ cells, and pre-treatment bone marrow histopathology are mandatory to stage the MPNs ET, PV, PMGM for proper prognosis assessment and therapeutic implications. Myelofibrosis (MF) itself is not a disease because reticulin fibrosis (RF) and reticulin/collagen fibrosis (RCF) are secondary responses of activated polyclonal fibroblasts to cytokines released from the clonal myeloproliferative granulocytic and megakaryocytic progenitor cells in ET.MGM, PV and PMGM.

[29] The erythrocyte count is a discriminative parameter between essential thrombocytopenia and polycythemia vera in JAK2V617F positive myeloproliferative neoplasm

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Abstract
Bone marrow morphology of clustered pleomorphic megakaryocytes and bone marrow cellularity on histopathology evaluation are overlapping in normocellular ET (WHO-ET), prodomal PV and PV carrying the JAK2V617F mutation. Bone marrow histopathology on its own is not reliable to differentiate between ET and PV and increased erythrocyte counts at a cut off level of 6x1012/L separates ET and prodomal PV from overt PV obviating the need of red cell mass measurement to distinguish PV from ET. Pleomorphism of megakaryocytes becomes more dysmorphic in advanced PV and ET.MGM as bone marrow cellularity, myelofibrosis and JAK2V617F mutation load increase during long-term follow-up. The characteristic features of prefibrotic JAK2V617F mutated trilineal myeloproliferative neoplasms (MPN) can be defined as a broad biological continuum of the earliest benign stage of normocellular ET, ET with features of polycythemia vera (prodomal PV), and hypercellular ET due to megakaryocytic granulocytic myeloproliferation (ET.MGM) followed by post ET and post-PV myelofibrosis. The WHO and European Clinical, Molecular and Pathological (WHO-ECMP) criteria distinguish JAK2V617F positive normocellular ET from JAK2 wild type normocellular ET carrying the MPL515 mutation and separates JAK2V617F positive prefibrotic ET. MGMT from JAK2/MPL wild type prefibrotic primary megakaryocytic granulocytic myeloproliferation (PMGM).

[30] JAK2-V617F Mutation Combined with Philadelphia Chromosome-Positive Chronic Myeloid Leukaemia

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Myeloproliferative neoplasms (MPNs) such as polycythemia vera, essential thrombocytopenia, primary myelofibrosis and chronic myeloid leukemia have too similar and accurate way to differentiate there is study of genetic disorders in these patients. Philadelphia chromosome is a sure way to definitely diagnose CML. Recently, JAK2V617F mutation introduced as a diagnostic marker for other myeloproliferative neoplasms. Many studies show that the absence of the JAK2 mutation in chronic phase Philadelphia positive CML. In contrast with these reports, more recently, several cases with the coexistence of Philadelphia positive chromosome and JAK2V617F mutation in blood and bone marrow samples were reported. Here, we report a patient that have the Philadelphia chromosome disorder and JAK2V617F mutation in same time.
Objectives and background

Essential thrombocythemia (ET) is a myeloproliferative neoplasm which does not substantially limit life expectancy, but it can seriously impact quality of life mainly due to thrombotic complications. Target drugs (Janus kinase and telomerase inhibitors) have the potential to change the course of the disease, but their safety should be evaluated in comparison with conventional therapy. The information about historical control on population basis could be valuable to assess potential benefits and additional costs of new diagnostic and therapeutic options. The objective of our study was to review the 10 year experience in managing ET in our centre.

Methods

Our institution serves as primary haematological outpatient department for about 2.4 million inhabitants. We reviewed patients’ charts to obtain the information about incidence, symptoms, diagnostic methods, results, treatment options and their relationship to prognostic factors. For thrombosis risk stratification, we used the WHO IPSET-thrombosis system. Statistical methods included descriptive and Kaplan-Meier methods.

Results

From 2003 to 2012, there were 218 newly diagnosed ET patients (161 females, 57 males). This yield incidence varied from 0.60 to 2.10 with a mean of 1.30 new patient per 100,000 inhabitants annually. The median age of the population was 57 years (range, 18 – 89). The most prevalent initial disease symptoms were: Fatigue (34.4%), headache and dizziness (27%), arthralgia (22%), erythromelalgia (17%), pruritus (7%). 85 (39%) patients were referred to haematologists only with elevated platelets (PLT) in complete blood counts (CBC). Diagnostic CBC data (mean (95%, CI)) were as follows: HB 14.0 (13.7-14.2) g/dl, WBC 9.9 (9.4-10.4) x 10^9/l, PLT 919 (869-970) x 10^9/l. Bone marrow fibrosis grade 0 was noted in 90.9%, grade 1 in 9.1% of patients. Cytogenetic abnormalities were seen in 7/75 (9.3%) patients. JAK2V617F was detected in 79/136 (58%) patients. The MPLW515L mutation was revealed in one patient. Thrombotic complications occurred in 67 (31%) of patients: 46 arterial and 29 venous thrombotic episodes, myocardial infarction in 22 (10%), cerebrovascular accident in 28 (13%) patients. WHO IPSET-thrombosis system showed significant (p<0.05) differences between risk groups. Patients’ risk stratification and thrombosis rates are presented in the table.

Conclusions

ET is one of the haematological malignancies with good life expectancy but with potentially serious impact on the quality of life. Thrombosis risk stratification systems had high predictive value in routine practice. Innovative drugs should be evaluated in comparison with historical control to assess the cost-benefit ratio.

References

Objectives and background
Myeloproliferative neoplasms (MPNs) have been associated with a high incidence of thrombosis and bleeding episodes, which significantly contribute to disease-related morbidity and mortality. Clinical data indicate an association of the JAK2V617F mutation, seen in nearly all polycythemia vera (PV) cases and almost 60% of those with essential thrombocythemia (ET) and myelofibrosis (MF). The mutation is also seen in 37% of patients with splanchnic vein thrombosis (SVT) and its presence was associated with an increased risk for SVT. However, the prevalence of JAK2V617 seems to be low in patients with other thromboembolic events in unusual sites such as cerebral sinus, upper limb deep venous thrombosis (DVT). Additionally, activating mutations of MPL gene, seen in 3% of ET and 5% of MF patients, are considered as a significant risk factor for microvessel disturbances and have been associated with an increased risk of arterial thrombosis. Retinal vein occlusion (RVO) is a thrombotic complication in an uncommon site that may result in sight threatening disease. In this study we investigated the prevalence of JAK2V617F and MPLW515L/K mutations in a prospectively assembled cohort of patients with RVO, hypothesizing that some cases may be associated with an underlying undiagnosed MPN.

Methods
We studied 52 (23 males and 29 females) consecutive patients with no evidence of an underlying MPN who had been diagnosed with RVO confirmed with fluorangiography from January 2007 to September 2011. The mean age was 70 years (range: 49-85) Twenty eight patients (53.8%) presented with central RVO and 24 patients with branched RVO (46.5%). DNA was extracted from peripheral blood samples by standard procedures. The JAK2V617F mutation was detected using a tetra-primer amplification refractory mutation system (ARMS) polymerase chain reaction (PCR) assay with a sensitivity of 1% and the allele burden was estimated with a semi-quantitative method. MPLW515L/K were detected using allele-specific PCR (AS-PCR) assays with a sensitivity of 1%.

Results
Overall, MPN associated mutations were detected in 5/52 cases. JAK2V617F was detected in 2/52 cases (3.8%; 95%CI-1.4%-9%), while MPL exon 10 mutations were detected in 3/52 (5.7%; 95%CI-0.6%-12%). The JAK2V617F allele burden in the two positive patients was 45% and 52% respectively. Both patients who carried the JAK2V617F mutation were female. The first patient had been already diagnosed with ET according to the WHO criteria at the time of RVO screening. She was receiving hydroxyurea and aspirin and her platelet count was normal. The second patient who also carried the JAK2V617F mutation had a PLT count of 850,000/μl at the time of screening and was diagnosed with ET within the 3 following months. The patients with MPL mutations presented with normal blood counts.

Conclusions
Our findings indicate that a latent MPN could underlie RVO even in the absence of conventional diagnostic criteria. Our results represent the first report that MPL mutations could underlie RVO cases and suggest that routine screening of RVO cases for MPN mutations may be useful, especially in older patients.
[33] Treatment of elderly patients with AML adjusted for the presence of comorbidities and performance status: Study of the Polish Adult Leukemia Group (PALG)


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Objectives and background
Despite the standard intensive chemotheraphy (IC) is deemed the best option for treatment of elderly patients with acute myeloid leukemia (AML), the majority of them are excluded on account of presence of comorbidities and performance status (PS). The Polish Adult Leukemia Group (PALG) conducted a prospective study to estimate the treatment outcomes in a group of elderly AML patients aged 60 years or more with different treatment approaches depending on the most important and approved prognostic factors such as comorbidities and PS.

Methods
Patients (n=537) with newly diagnosed AML were stratified according to the modified Charlson Comorbidity Index (CCI) and ECOG PS into fit and frail groups. The fit patients were characterized by CCI ≤2 and ECOG ≤2 and they were treated with intention to induction remission. Those with CCI 0 received standard IC and patients with CCI 1-2 received reduced-intensity chemotherapy (RIC). The frail patients with CCI>2 and ECOG PS>2 obtained the best supportive care with cyclotherapeutic chemotherapy if appropriate, but without intention to induction remission.

Results
The fit patients CCI 0, fit patients CCI 1-2 and frail patients, respectively, demonstrated complete remission (CR) rates of 35%, 22% and 0%, medians of overall survival (OS) of 39, 26 and 14 weeks, and 8-week mortality rates of 31%, 24% and 31%. Despite there was a statistically significant difference between the CR ratio (p=0.008) in the fit CCI 0 and CCI 1-2 subgroups, the probability of OS was similar in both subgroups. Using a multivariate regression analysis, in the fit CCI 0 subgroup, no factor had an impact on CR achievement. However, in the fit subgroup CCI 1-2, unfavorable karyotype and WBC>10 G/L turned out to be independent risk factors for achieving CR. The independent factors influencing the probability of 8-week mortality were WBC >10 G/L (in the fit subgroup CCI 0), age >75 years (in the fit subgroup CCI 1-2), and WBC >10 G/L (in the frail group).

Conclusions
The present study suggests that the classification of fit patients with ECOG ≤2 and presence of comorbidity (CCI 1-2) to R-IC have enabled the enlargement the group of elderly AML patient who may be treated with intention to induction remission and the application of chemotherapy with intensity where the benefit/risk ratio is the most suited to the individual patient.

[34] Spliceosome gene mutations are rare in acute promyelocytic leukemia

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Objectives and background
Acute promyelocytic leukemia (APL) is a distinct subtype of myeloid leukemia which is characterized by typical chromosomal translocation between chromosomes 15 and 17. This translocation produces a fusion product of the promyelocytic leukemia gene (PML) and the retinoic acid receptor α (RARA). Due to this mechanism, APL has the best prognosis of all AMLs because it can be treated with regimens containing a targeted therapy with all-trans retinoic acid (ATRA), which counteracts the PML-RARA induced pathomechanism. Despite of this, approximately 10% of APL patients show relapse depending on their individual risk profile, and it is hypothesized that APL cells carry further genomic alterations that cooperate with PML-RARA. Recent studies have identified novel heterozygous mutations in genes of the spliceosomal apparatus in myeloid malignancies. Mutations of spliceosomal genes in APL have not been investigated in larger cohorts.

Methods
Genomic DNA from APL bone marrow blasts of 48 patients (21 male, 27 female) at initial diagnosis was analyzed. Mean age was 48 years (range 12-78). Direct Sanger sequencing was carried out to screen for mutations in known hotspots of spliceosomal genes: SF3B1 exon 14 (mutational hotspots K700E and H662Q), exon 15 (mutational hotspot K700E), U2AF1 exon 2 (mutational hotspot P95H) and SRSF2 exon 1 (hotspot S34F/Y). Data analysis was carried out with Geneious Pro software, vers. 6.05.

Results
By screening 48 APL patients for common mutations in genes of the spliceosome, one K700E mutation of SF3B1 could be detected in one patient. The patient carrying this mutation also had a FLT3 D835 mutation and an unbalanced t(15;17) as determined by high density SNP array. Treatment of this patient consisted of the AMLCG > 60 protocol. The patient achieved a complete remission with duration of CR with a follow up beyond 12 years. All other patients were negative for the interrogated mutations of spliceosomal genes.

Conclusions
Since the only mutation in 192 sequence analyses of splicing gene mutational hotspots in 48 APL patients was one K700E mutation, it can be concluded that spliceosomal gene mutations in APL are rare and do not play an important role in its molecular pathogenesis. However, the recently available deep sequencing may reveal so far unknown mutations in spliceosomal genes.
[35] Clinical, hematological and molecular-genetic variability of acute myeloid leukemia (AML) with aberrant CD7 expression on blast cells

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Objectives and background
Flow cytometry has become an important diagnostic tool for the classification of AML; it can detect minimal residual disease in the post-remission period, predict responses to induction therapy, and evaluate the risk of relapse and overall survival (OS). In most AML cases, aberrant antigen expression is recognized as a poor predictor; meanwhile its prognostic value remains controversial with contradictory results. Approximately 10-40% of AML patients are characterized by aberrant expression of CD7 on the surface of myeloblasts. However, there have been conflicting data regarding a correlation between CD7 expression and disease outcome. It is some publications it has been demonstrated that CD7 expression is associated with low remission rates and decreased OS. So it is questionable whether CD7-positive tivity can be described as an independent prognostic factor or whether it is a casual finding, connected with clinical, hematological and molecular-genetic factors (cytogenetics, age, leukocytosis, mutations). The aim of the study was to determine the heterogeneity of AML patients with aberrant CD7 expression. The objective was to assess CD7 expression in patients with AML, to evaluate its correlation with the different clinical and laboratory data as well as its relation to disease outcome.

Methods
For immunophenotyping, five-color flow cytometry was performed using a panel of monoclonal antibodies. CD7 was regarded positive when at least 20% of gated cells were more fluorescent than the isotype-matched negative control. Standard G+T-method was used for karyotyping. Mutations in FLT3 and NPM1 were detected by polymerase chain reaction (PCR). To form the investigational group, the data of 132 patients with de novo AML were analyzed.

Results
After 3-phase selection, 31 patients (23.5%) were included into the investigational group. The median age of patients was 55 years. 17 patients had normal karyotype (NK), 11 had single aberrations, 2 patients had two independent aberrations, and 1 patient had a complex karyotype. Mutations were detected in 13 cases: FLT3-ITD in 9 patients, FLT3-TKD in 2 patients and NPM1 in 3 patients. After standard induction chemotherapy “7+3” complete remission was detected in 17 patients. Patients whose myeloblasts were characterized by high level CD7 expression (≥70%) were older than patients with lower expression: 57.5 vs 44 years, respectively; p=0.011. We did not find any significant association between CD7 expression and OS. At the same time, there was a relation of OS with age and mutations (univariate analysis). Using multivariate analysis, we did not prove the value of age and molecular-genetic damages as independent prognostic markers; p=0.363 and p=0.084, respectively. But there was a tendency to OS decrease in patients with co-expression of CD7 and FLT3-ITD or unfavorable cytogenetic aberrations.

Conclusions
Morphological, cytogenetic and molecular heterogeneity of AML with CD7 co-expression was revealed. CD7 expression in AML blasts is not an independent prognostic factor; meanwhile it does not eliminate the value of antigen expression (simultaneously) as risk marker. These data indicate that when studying the prognostic value of CD7 in AML, the cytogenetic status, mutations and the age of the study population significantly skew the results.

[36] Immunophenotypic and clinical features of acute myeloid leukemia patients with normal cytogenetics and mutation of the FLT3 gene

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Objectives and background
Acute myeloid leukemia (AML) is a heterogeneous disorder that results from a block in the differentiation of hematopoietic progenitor cells along with uncontrolled proliferation. Specific chromosomal aberrations can be identified in approximately 60% of cases and are the most important tool to stratify patients into prognostic groups. The largest subgroup with normal karyotype (NK) is classified as intermediate risk. The optimal therapeutic strategies for these patients are still largely unclear. Risk stratification for NK-AML patients may be possible due to immunophenotypic and molecular results. The goal of our research was to determine distinctive features of de novo acute myeloid leukemia with FLT3-ITD mutation. The objective was to review the variability of morphological and cytogenetic characteristics of AML patients with FLT3-ITD; and to identify homogeneity of the group of AML patients with NK with FLT3-ITD based on hematological data and the immunophenotype of blast cells.

Methods
Standard G+T-method was used for patients karyotyping. Mutations in FLT3 and NPM1 were detected by polymerase chain reaction (PCR). For immunophenotyping, five-color flow cytometry was performed using a panel of monoclonal antibodies. The immunophenotypic group included 101 AML patients. FLT3-ITD mutation was detected in 21 cases (20.8%). Meanwhile, 13 patients (61.9%) from this group had NK. Simultaneously with FLT3-ITD, three patients had NPM1 mutations. The group of patients with expression of several CO markers more than 20% (diagnostically significant number) was analyzed.

Results
There were no significant differences in the number of patients with high expression of CD34 in the control group and the group with FLT3-ITD: 83.3% vs 90.0%; p=0.635. At once, the number of patients with high expression of HLA-DR and CD7 was significantly higher in group with NK and FLT3-ITD: 6.2% vs 50.0%; p=0.007 and 55.6% vs 100.0%; p=0.014, respectively. Correlation analysis showed FLT3-ITD to be determined significantly often in patients older than 51 years in comparison with young; r=0.400; p=0.034.

Conclusions
AML with NK and FLT3-ITD represent the group, homogeneous on a number of hematological data and biological characteristics of leukemic cells. Thereby, such markers as leukocytosis in combination with aberrant expression of HLA-DR and CD7 on blast cells allowed prognosticating the probability of detection FLT3-ITD mutation in AML patients with NK. This information can be used as a prevention of relapse and expansion of relapse-free survival due to early prescription of FLT3 inhibitors. Thorough analysis of the complex of immunophenotypic, cytogenetic and molecular results allows to establish the nature of blast cells and thus to choose a suitable scheme of treatment.
Objectives and background
Sex chromosomes are infrequently involved in patients with hematologic malignancies. In most instances, the abnormality is either duplication in the q arm or deletion and translocation involving the q13 and q24 regions.

Results
We report herein a rare translocation t(X;10)(p10;p10) in a newborn with 2 months and 20 days with acute myeloid leukemia (AML) (FAB, M4). Cytogenetic analysis detected a cell clone with t(X;10) (p10;p10). This was confirmed by FISH analysis with whole chromosome painting (WCP) specific for chromosomes X and 10. The patient was treated with chemotherapy, and a complete morphologic and cytogenetic remission was achieved. To our knowledge, our case is the first report of a neonatal AML with t(X; 10). The patient had an excellent early response to a salvage AML-type therapy. The prognostic significance of the t(X; 10) in this setting remains unclear.

Conclusions
Due to the rarity of this translocation, further cytogenetic and molecular biologic studies are required to elucidate the clinical and molecular significance of this unusual karyotypic finding.
Objectives and background
Hematologic disorders as myelodisplastic syndrome (MDS) and post hematopoietic stem cell transplantation (p-HSCT) are characterized by severe anemia with high transfusion requirements and secondary iron-overload. The efficacy of deferasirox (DFX) and deferoxamine (DFM) as chelators on these diseases has been studied during the QUELAFER study in 2011-2012 (EUDRACT: 20009017799-26). Here we present the outcomes of patients after +12 months on follow-up. The aim was to analyze the impact of chelation therapy on patients’ outcomes after +12 months after the study finished, comparing the iron pattern evolution.

Methods
Using the QUELAFER-study database, we selected the patients with hematological malignancies who completed the study period and registered the follow-up data regarding their outcomes on iron pattern and clinical status at 6, 12, 18 and 24 months after study finished.

Results
A total of 16/27 patients with hematological malignancies completed the study and were included for analysis. Mean age: 58.6 (31 – 77); male/female ratio: 7/9; diagnosis with MDS: 12 patients; p-HSCT: 4 patients. Seven patients with mutations in the HFE-gene; one homozygous with H63D; 4 heterozygous with H63D/N; and 1 with S56C/N. Eight patients entered each arm and received DFX (Arm-A) and DFM (Arm-B). Baseline-QUELAFER serum ferritin (SF) was 1009 ng/dL (635-1458) and liver iron concentration index (LIC) 100.095 μmol/g; end-study SF was: 527 μmol/g (155-1090) and LIC: 44.1 μmol/g for Arm-A, and 680 (312-1395) ng/dL and LIC: 67.2 μmol/g for Arm-B. Follow-up: At 6 months, SF was 880 ng/dL for Arm-A and 1018 ng/dL for Arm-B; at 12 months SF levels were: 415 ng/dl and 1017 ng/dL; at 18 months SF levels were 402 ng/dL and 498 ng/dL, respectively. In Table 1, we show the iron pattern evolution per patient. It is remarkable that 7/8 patients in Arm-A have been with SF levels below 1000 ng/dL during at least 1 year and none of 8 have re-started chelation therapy; on the other side, 1 patient on Arm-B started chelation therapy with DFX at 6 months for increased SF levels. At this moment, 2 patients had died, one in each arm; one p-HSCT Arm-B patient with bad response to DFM developed a severe pulmonary infection. The rest of patients have improved their hematologic conditions clinically. None of patient had needed transfusion support.

Conclusions
In our small cohort of patients with iron overload, we observed a significant reduction of SF levels and a reduction on LIC values. The follow-up time showed an improvement on clinical conditions and stable iron levels below 1000 ng/dL, especially on patients treated with DFX.
[39] Diagnostic usefulness of flow cytometric detection of altered neutrophil and monocyte maturation patterns in myelodysplastic syndromes

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Objectives and background
Myelodysplastic syndromes (MDS) are a group of disorders for which standard criteria for diagnosis, based on clinical history, morphology, histology and cytokinetic data are established. However, in a significant number of cases, morphology and cytogenetic studies are not fully diagnostic. Flow cytometry (FCM) immunophenotyping has been proposed as a method to improve the evaluation of marrow dysplasia. The aim of our study was to evaluate the usefulness of detection by FCM immunophenotypic (IF) aberrancies and altered maturation patterns of granulocytes and monocytes for identification of myelodysplastic syndromes.

Methods
An analysis by FCM of bone marrow cells was performed in addition to routine cytogenetic analysis and morphological evaluation of marrow dysplasia in cytopenic patients with suspected MDS. This analysis was performed since January 2008 in the Flow Cytometry Laboratory of the Department of Hematology of Poznan University of Medical Sciences. Bone marrow samples were collected on EDTA. The samples were stained as a part of routine clinical flow cytometric testing with the four-color directly conjugated antibody combinations. The standard stain-lyse-wash method was used. Cells were acquired on the day they were stained using a FACSCalibur flow cytometer (BD Biosciences). CellQuest software (BD Biosciences) was used for analysis. Granulocytes, monocytes and lymphocytes were gated on a CD45/SSC and CD45/CD71 display. The expression of CD34, CD117, CD13, CD33, CD11b, CD14, CD15, CD56 and lymphoid antigens on neutrophil and monocytes were evaluated, as well as maturation patterns based on plots of CD13 vs. CD16, CD33 vs. CD117 and CD11b vs. HLA-DR.

Results
The study group consisted of 86 cytopenic patients with suspected MDS. Final diagnosis of MDS based on standard criteria was established in 53 patients. In 33 patients, MDS was excluded as a cause of cytopenia. In the group of patients with confirmed MDS in comparison with the group with excluded MDS, the abnormal maturation patterns were detected more frequently on the plots of CD33 vs. CD117 (41% vs. 12%; p=0.007), and CD11b vs. HLA-DR (31% vs. 3%; p=0.002). Additionally, in the group of patients with MDS, the abnormal CD56 expression was found more frequently on both neutrophils (23% vs. 0%; p=0.003) and monocytes (80% vs. 33%; p=0.028). Based on these results, for each patient, a simplified FCM score was calculated as a sum of detected IF aberrancies (from 0 to 4). In a significantly higher proportion of patients with MDS in comparison with patients with other final diagnosis, simplified FCM score higher than 1 was noticed (47% vs. 0%; p<0.001). Positive and negative predictive value of simplified FCM score >1 was 100% and 61%, respectively. Moreover, in the group of patients with MDS, the positive correlation between simplified FCM score and International Prognostic Scoring System (IPSS) was found (p=0.044).

Conclusions
Flow cytometric detection of aberrant neutrophil maturation patterns on the basis of CD33 vs. CD117 and CD11b vs. HLA-DR plots analysis, as well as abnormal CD56 expression on neutrophils and monocytes, is useful in diagnosing MDS. The correlation between the numbers of IF abnormalities and IPSS suggests a prognostic value of proposed simplified FCM score.

[40] Diagnostic and differentiating value of selected dysplastic features of granulocytes in myelodysplastic syndromes

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Objectives and background
Dysplastic changes in granulocytes and their precursors appear in blood cells and bone marrow in various neoplastic and non-neoplastic diseases. Appearance of above changes on the level of ≥ 10% became a diagnostic criterion in myelodysplastic syndrome (MDS). It is not defined which changes have diagnostic and differentiating value of a particular subtype of myelodysplastic syndromes. The heterogeneity of dysplastic changes—a key topic of this study—refers to both, nucleus and cytoplasm of granulocytes. The objectives of this retrospective analysis are as follows: a) frequency of granulocytes dysplastic changes in selected subtypes of MDS, b) assigning a set of dysplastic changes specific for particular subtypes of MDS and setting of excluding changes, c) determination of co-existence of various dysplastic features in a single cell and its diagnostics and differentiating value.

Methods
Archive bone marrow smears of 176 patients (106M/70F) were assessed; patients had an average age of 75.0 yrs, with the following subtypes of myelodysplastic syndromes: RCMD – 103 (58.5%), RARS – 11 (6.3%), RAEB1 – 35 (19.9%), RAEB2 – 27 (15.3%). From the most often described and acknowledged features of granulocytes dysplasia, the following were selected: Vacuolated cytoplasm, vacuolated nucleus, hypersegmented neutrophils, agranular cytoplasm, toxic granulation, giant cell size (myelocyte, metamyelocyte and band), pseudo-Pelger-Huet anomaly, abnormal division shape, abnormal nuclear shape (ring), bizarre nuclei, dense chromatin (clumping chromatin). Auer rods, Döhle bodies and apoptotic bodies. The analysis was performed on 300 granulocytes and their precursors by 2 independent cytomorphologists. The next step was the assessment 500 consecutive marrow cells to confirm myelodysplasia. The analysis was performed in the STATISTICA9.1 PL software.

Results
The most frequent features of granulocytes dysplasia in all subtype of MDS are: Vacuolated cytoplasm (92%), agranular cytoplasm (82.4%), vacuolated nucleus (75.6%), toxic granulation (41.3%) and giant cell size (42%), the other features of dysplasia were below 25% (3.4-23.9%). The median of numbers of dysplastic cells with vacuolated cytoplasm and nucleus were in RAAS 1.68% and 0.45%, in RCMD 5.76 and 2.04%, in RAEB1 1.83% and 3.45%, and in RAEB2 9.64% and 4.66%, respectively. The average value of dysplastic cells with agranular cytoplasm was 13.65% in RCMD, 14.12% in RARS, 13.91% in RAEB-1 and 16.86% in RAEB-2. The median of numbers of dysplastic cells with other features of dysplasia were below 1%. It showed statistical significance for assessed features (p<0.05). The average rate of dysplasia was 31% and was similar for all subtypes of MDS.

Conclusions
• Frequent features in all subtypes of MDS such as vacuolated cytoplasm, vacuolated nucleus, agranular cytoplasm and toxic granulation disclose diagnostic value of granulocytes dysplasia.
• Features of dysgranulopoiesis such as pseudo-Pelger-Huet anomaly, hypersegmented neutrophils, abnormal nuclear shape (ring), bizarre nuclei, Auer rods and apoptotic bodies do not show diagnostic value because they appear in the average proportion of cells under 1% of studied diseases.
• Differentiating value of features of granulocytes dysplasia was seen only in single subtype of MDS. In RAEB: low percentage of cells with vacuolated cytoplasm and nucleus; in RAEB1 and 2: high percentage of cells with vacuolat ed cytoplasm and nucleus.
• Analysis of the features of dysplasia does not allow to calculate a dysplasia index which would have differentiation value.
[41] Impact of bone marrow cellularity on disease characteristics and prognosis in myelodysplastic syndromes

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Objectives and background
Although 10% of all MDS patients present with hypocellular bone marrow, not many studies have taken bone marrow cellularity into consideration so far. Still, questions concerning prognostic and outcome of hypocellular MDS patients remain unanswered. Therefore, we performed an analysis on bone marrow cellularity, its influencing factors and possible consequences on patients' prognosis.

Methods
In our study, we focused on MDS patients who underwent both bone marrow aspirate and biopsy. As a biopsy allows a more informative insight on the bone marrow structure, we concentrated on data of patients with histologically examined marrow and analysed hypocellular (n=323) as well as normocellular (n=956) and hypercellular (n=953) cases of the Düsseldorf MDS registry. We also included results of peripheral blood counts (HB, WBC, PLT, LDH, blasts etc.), cytogenetics and prognostic scores like IPSS, IPSS-R, WPSS and MDACC score.

Results
Factors like age, gender, HB, platelets and LDH did not show any significant differences between the three types of cellularity. Hypocellular patients present with significantly lower ANC values, when compared to the other groups (p=0.001). Hypocellular MDS have a higher tendency to develop after previous chemotherapy or radiation, as well as for hypocellular secondary MDS cases are significantly higher than for normo- and hypercellular MDS (12.3%; 5.1%; 6.6%; p=0.001). Bone marrow blasts <5% occur in hypocellular cases (55%) more often than in hypercellular MDS (50.3%), but less than in normocellular cases (64.4%) (p<0.0005). With regard to WHO subtypes, hypocellular cases present as RCUD more often, when compared to hypocellular bone marrow. Our analyses revealed the fact that hypocellular MDS has higher rates of monosomy 7, especially in comparison with normocellular MDS. Rates for hypercellular cases did not differ much. Tq-mutations even have an even higher association with hypocellular than with monosomy 7 indeed.

Conclusions
Although hypocellular MDS patients do not present with significant differences in blood counts, we could clarify that hypocellular MDS patients show distinct differences in bone marrow blasts, WHO subtypes, cytogenetic aberrations, secondary MDS and prognosis. Potentially, immunologic effects may play a role in the pathophysiology of hypocellular MDS.

[42] Clinical significance of splicing gene mutations in MDS and secondary AML in the setting of allogeneic hematopoietic stem cell transplantation

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Objectives and background
Mutations in the splicing gene machinery have been described as frequent aberrations in MDS. The aim of this study was to investigate the diagnostic impact of mutations in the splicing genes U2AF1, SRSF2 and SF3B1 in a large cohort of patients with high-risk MDS or secondary AML following MDS (sAML) undergoing allogeneic HSCT.

Methods
Patients (n=339) with a diagnosis of MDS (50.1%) or sAML (49.9%) who received allogeneic HSCT at four German University Medical Centers between 1996 and 2011 were evaluated for the presence of mutations in the splicing genes U2AF1, SRSF2, and SF3B1 by direct sequencing.

Results
Median follow-up from time of transplantation was 3.27 years. Low, intermediate, and high-risk cytogenetics according to IPSS were found in 204 (60.2%), 42 (12.4%), and 76 (22.4%) patients, respectively (in 5%, cytogenetic information was not available). Related donor HSCT was performed in 82 patients (24.2%), and unrelated donor HSCT in 257 patients (75.8%). Mutations in U2AF1, SRSF2 and SF3B1 were detected in 14 (4.1%), 32 (9.4%) and 18 (5.3%) patients, respectively. Baseline characteristics were similarly distributed between U2AF1, SRSF2, or SF3B1 mutated and wild-type patients, respectively (sex, MDS vs sAML, cytogenetics, CMV status of patient, type of previous treatment, and remission status prior to transplantation), except a higher median age of U2AF1 mutated compared to wild-type patients (p=0.02). There were no differences regarding transplant-related characteristics between patients with mutated or wild-type U2AF1, SRSF2, and SF3B1. U2AF1 mutations were associated with a significantly shorter overall survival (OS, median 0.58 vs 3.6 years in mutated vs wild-type patients, respectively, HR 2.54; 95%CI 1.41-4.58; p=0.002). The cumulative incidence of relapse (CIR) was higher in U2AF1 mutated compared to wild-type patients (5-year CIR 50% vs 22%, p=0.002), while non-relapse mortality (NRM) was similar between mutated and wild-type patients (3-year NRM 43% vs 29%, p=0.11). Mutations in SRSF2 and SF3B1 were not associated with OS (p=0.98 and p=0.44, respectively), CIR (p=0.19 and p=0.19, respectively), and NRM (p=0.49 and p=0.44, respectively). In multivariate analysis, when considering variables with p<0.15 in univariate analysis, U2AF1 mutations independently predicted shorter OS (HR 2.6; 95%CI 1.39-4.85; p=0.01) besides karyotype, stage, CMV serostatus, and donor sex. Mutations in U2AF1 independently predicted higher CIR in multivariate analysis (HR 3.02, 95% CI 1.36-6.7, p=0.007).

Conclusions
In conclusion, U2AF1 mutations independently predicted worse patient outcome after allogeneic HSCT in MDS and sAML patients in our study due to a higher incidence of relapse.
In spite of recent progress, therapeutic options for myelodysplastic syndromes (MDS) are still limited. Enhanced progenitor proliferation, bone marrow (BM) hypervascularization and disturbed immune regulation are known to contribute to MDS pathogenesis. As mammalian-target of rapamycin (mTOR) is a key regulator involved in these processes, inhibition of this complex might be a promising strategy. We report on the effects of single agent temsirolimus (TEM) on the clinical course as well as on immune composition and BM vascularization of MDS patients treated within the prospective, multicenter “TEMDS”-trial (NCT01111448) of the German MDS study group.

**Methods**

A total of twenty patients presenting either IPSS low/int-1 MDS (n=9) or IPSS int-2/high after azacitidine failure were included. Participants received weekly TEM at a dose of 25 mg for a maximum of twelve 4-week cycles in responding patients. Translational research within this study encompassed flow-cytometry-based measurement of changes in T-cell composition as well as determination of cytokine levels and BM-vascularization prior to and after TEM.

**Results and Significance**

Only 5 of 20 patients treated reached the pre-specified response evaluation after cycle 4, while 15 discontinued TEM treatment prematurely due to intolerable side effects (n=11), infectious complications (n=3), or progression to AML (n=1). A total of 13 serious adverse events mainly of infectious origin were encountered in 10 patients and 1 patient died during TEM treatment. None of the 5 patients who were treated for at least 4 months responded to treatment according to IWG criteria. Bone marrow hypervascularization decreased significantly (p=0.006) in a total of 12 assessable patients (Fig. 1A) although there was no indication that these changes were related to the medullary (Fig. 1B) or peripheral blood VEGF concentration. Total lymphocyte count in the pB (pre: 74.6%, post: 48.4%, p=0.083) and BM (pre: 23.5%, post: 20.1%, p=0.123) decreased substantially. Within the T-helper cell compartment a trend towards an increase in regulatory T-cell (Treg) frequency was observed (pB: pre: 6.0%, post: 6.4%, p=0.083). Moreover, the balance between naive (CD45RA+/CD45RO-) and activated/memory (CD45RA-CD45RO+) Treg shifted significantly in favor of the latter (p=0.004). Plasma analysis in BM and pB revealed that these changes were obviously not mediated by alterations in TGFβ plasma levels (data not shown).

**Conclusions**

The mTOR-inhibitor temsirolimus reduces BM hypervascularization and alters T-cell composition in MDS patients. However, these effects do not seem to alter the natural course of the disease and treatment is accompanied with severe side effects.