Abstracts

ELN Frontiers Meeting 2014
Where Science Meets Clinical Practice
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Chairs: Michele Baccarani
Rüdiger Hehlmann

Co-Chairs: Francisco Cervantes
François Guilhot
Wolf-Karsten Hofmann
Gert Ossenkoppele
Dear Colleague,

Welcome to the 8th edition of the ELN Frontiers Meeting, themed “Where Science Meets Clinical Practice”, in Berlin, Germany. It is our privilege and honour that so many haematology fellows from more than 50 countries worldwide have taken the opportunity to participate in this educational event.

The organising committee, building on the success of previous years, has structured a 2.5 day programme covering chronic myeloid leukaemia (CML), myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML). We hope you all find the discussed topics informative, challenging and exciting.

A primary focus of the meeting is to provide a platform for young investigators to showcase their own research. We would like to extend our thanks to all attendees who submitted an abstract to be published in this special edition of the ELN newsletter. We are pleased about the high quality, the novelty, and importantly, also the clinical relevance of many abstracts. We are also grateful to all co-chairs who assisted in the peer-review to assess the submitted work.

As usual, we have received far more abstracts than we have slots for presentation in the main programme. We congratulate all those authors that have been chosen to present their work orally, and we anticipate exciting discussions in the parallel poster presentation sessions and in the poster walks throughout the ELN Frontiers Meeting 2014.

We are glad you were able to come and wish you an enjoyable, successful and stimulating conference.

Yours sincerely,

Rüdiger Hehlmann
Michele Baccarani
CML ABSTRACTS

[1] DRESS (Drug reaction with eosinophilia and systemic symptoms) after treatment by Imatinib

[2] Droplet digital polymerase chain reaction (ddPCR) assessment of deep molecular response in Chronic Myeloid Leukemia (CML)

[3] Exceeding MR5.0 sensitivity in routine BCR-ABL1 analysis using multiplex ddPCR


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[54] Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) for adults with Acute Myeloid Leukemia (AML): the Tunisian results

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Boosting and sharing integrated Next Generation Sequencing, in vitro and in vivo approaches for targeted personalized therapy of rare tumors: from bench to bedside
Background and objectives

Imatinib (Gleevec) is a tyrosine kinase inhibitor used for treatment of chronic myeloid leukemia. We report a case of drug reaction with eosinophilia and systemic symptoms (DRESS) following treatment with imatinib.

Patient and methods

A 52 year-old women was treated with imatinib for chronic myeloid leukemia. A macular and pruriginous eruption appeared on the patient’s trunk seventeen days after starting medication with gradually extension. The patient was admitted for generalized prurigenous rash with facial edema, fever, thrill and occipital lymphadenopathy. Laboratory data showed hypereosinophilia. Histologic analysis of skin biopsy specimens suggested a drug –induced reaction. Imatinib was stopped and replaced with Nilotinib (Tasigna). Improved clinical and laboratory results were seen.

Discussion

DRESS (drug reaction with eosinophilia and systemic symptoms) is a drug-induced life-threatening hypersensitivity syndrome that presents with skin rash, fever, eosinophilia and multiple organ involvement. Drugs most frequently implicated are aromatic antiepileptic agents, sulfonamides and allopurinol. To our knowledge, this is the fourth case of DRESS following treatment with imatinib. Cutaneous reactions to imatinib are frequent and usually presented as a maculopapular eruption, and facial edema. Few cases of serious skin reactions have been reported until now. Several authors suggest that the prevalence and severity of cutaneous manifestations are related to a pharmacologic effect of imatinib.

References:


[2] Droplet digital polymerase chain reaction (ddPCR) assessment of deep molecular response in Chronic Myeloid Leukemia (CML)

Susanna Akiki1,3, Jane Bryon1, Manasi Jyothish2, Chris Campbell1, Susan Rose1, Kerry Wall1, Charles Craddock2,3, Manoj Raghavan2,3 and Mike Griffiths1

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3School of Cancer Sciences, University of Birmingham, UK

Background and objectives

Molecular monitoring of BCR-ABL1 transcript levels is central to effective clinical management of patients with CML. Evaluation of depth and durability of response assessed with standardised real time quantitative PCR (RT-qPCR) forms the basis of the European Leukemia Net (ELN) treatment recommendations 1.

Methods

A series of 50 patients previously determined to have achieved a major molecular response (MMR) were retrospectively monitored for BCR-ABL1 transcripts using ddPCR. Patients were selected from four broad categories: 1. Persistent negative patients with undetectable BCR-ABL1 transcripts for over 2 years, 2. Intermittent positive patients alternating between detectable, 3. Undetectable low level BCR-ABL1 transcripts and 4. Patients with molecular relapse who lost MMR.

Results

A comparison of BCR-ABL1 to ABL1 ratios obtained by ddPCR and RT-qPCR suggested three distinct clusters: 1. Patients with higher BCR-ABL1 transcript levels by ddPCR suggesting ddPCR may be more sensitive than RT-qPCR, 2. Patients who had higher BCR-ABL1 transcript levels by RT-qPCR suggesting either a greater sensitivity achieved using RT-qPCR or false positive variation, inherent in low level detection using RT-qPCR but not in ddPCR and 3. presumed outliers.

Conclusions

In conclusion this study demonstrates ddPCR has the potential to accurately discriminate between patients achieving a sustainable response who could be considered for treatment cessation studies from those in whom BCR-ABL1 transcripts are likely to re emerge.

References:

[3] Exceeding MR5.0 sensitivity in routine BCR-ABL1 analysis using multiplex ddPCR

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1Department of Clinical Immunology and Biochemistry, Vejle Hospital, Denmark

Background and objectives
Clinical studies are currently initiated where treatment of CML patients with tyrosine kinase inhibitors is stopped. One of the main criteria for patients to be eligible for these studies is that they must have achieved a molecular response of MR4.0. However, in order to obtain a reliable molecular measurement at the MR4.0 sensitivity level a higher sensitivity is needed in the analysis. We have tested a multiplex droplet digital PCR (ddPCR) approach in order to obtain reliable measurements at the MR5.0 level. When using a standard laboratory protocol with three reference genes and singleplex qPCR, only ¼ of the sample is tested for BCR-ABL1 transcripts. By using multiplex PCR the entire sample can be tested. The advantage of ddPCR is that an absolute concentration is measured and there is no need for calibration or standardisation of the analysis.

Methods
Blood samples stabilized in Paxgene tubes from 36 CML patients were used for the study. Samples were analysed according to the standard laboratory protocol with qPCR using B2M, BCR and GUSB as reference genes. For the study presented here RNA was purified from 5 million cells using the QiaSymphony RNA kit (Qiagen). 2.5 ug of RNA was used for cDNA synthesis using SuperScript VIVOL cDNA synthesis kit (LifeTechnologies). Samples were analysed with multiplex dd PCR on the QX100 system (Biorad). The WHO reference genes BCR and GUSB were used as reference. Eight wells were analysed for BCR-ABL1 (FAM labelled probe). Four of the wells were also analysed for BCR and four were analysed for GUSB (HEX labelled probes).

Results
The median number of GUSB transcripts in the samples was 605,000 copies (range 195,520-934,400). According to the EUTOS “Working definitions of Molecular Response in CML” (ref), MR5.0 sensitivity is obtained when analysing 240,000 GUSB transcripts. All samples except one were above this level. MR5.5 is obtained when analysing 759,000 GUSB transcripts and 20% of the samples (7/36) reached this level. The ddPCR results were compared to the qPCR results and a high concordance was seen in the positive samples (r2=0.94). 21 samples were positive by qPCR, and an additional 4 samples were positive by ddPCR.

Conclusions
Using multiplex ddPCR protocol developed 97% of the analysed samples had a sensitivity of MR5.0 or better. Compared to the labs routine qPCR protocol 20% more samples were found positive with ddPCR. Due to the multiplex setup of the ddPCR the entire cDNA sample can be tested for BCR-ABL1 transcripts. Contrary to qPCR there is no need for calibration of the ddPCR analysis and the method has potential for routine use when a high sensitivity is needed.

References:


Mohamed Azzazi1, Mervat Mattar2, Ashraf El handour1, Manal El Sorady1, Samir Shehata3, Rasha Ibrahim Mostafa1, Rasha Magdy1, Hend Ellithy2, Mohammad Shalby1, Rana khaled2, Mai Sameir1, Mohab Elshaer3

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Background
The first ALN report1 demonstrated that age-specific rates for CML in Egypt and Arab nations are lower by at least two decades compared to western populations (highest in age group 30-35 years). 1. Geographic and ethnic variations contribute to the variability of incidences among CML registries2. Reliable data concerning response rates to therapy in Arab nations is lacking. CML management areas are not in line with the current recommendations, due to sub-optimal timing of treatment decisions under monitoring, and lack of molecular techniques or 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 depending on the ease of access to medical services that show great diversity in AFME region1.

Objectives
1) To Release data of second ALN report of CML epidemiology in Egypt, after enrolling 6 new centres.
2) To investigate the low mean age of CML in Egypt.3) ACAs added to evaluate clonal evolution.

Methods
We analyzed data of 578 CML Egyptian patients (302 male and 276 female) followed-up for 5 years. Data were collected from 10 centers according to ELN2 and EUTOS recommendations2-3 by using a multicenter web based data registry portal, the ALN. (www. aln-afme.com). Cytogenetics: chromosome (ch) banding and FISH analysis at diagnosis. ACAs analysis: namely for Y-ch trisomy 8, duplication of Ph, isochromosome 17q, trisomy 19, and Deletion of der(9) ch.

Results
Patients Median age was 43y (42y for males, 43y for females), the age specific rates were highest for age group 30-35 years. At diagnosis 87% patients were in chronic phase(CP) CML, 8.1% in accelerated, and 4.9% in blastic phase. Sokal score: Low risk 57.8% Intermediate 24.5%

CML ABSTRACTS
and High in 17.7% BCR-ABL transcript level was performed to all cases, FISH to 87.4%. All patients received TKI therapy, (52% imatinib, 34% nilotinib 20% dasatinib and 4% needed therapies plus TKI). During 5 years 39.4% of patients achieved CR response, 89% PCYR, 72% CCRY, and 68% achieved MMR. Transplantation rate was 18% for females and 20% for males. Median survival /PFS were equal in female and male patients. We identified 54 CML cases with ACAs, they had inferior outcome, with lower cytogenetic and molecular response rates and longer response time to TKI. ACAs frequency was higher in younger patients, in Imatinib resistant and blast crisis patients.

Conclusions
The importance of ethnicity, socio-economic and gender differences in relation to disease incidence, diagnosis, and prognosis has been realized and became a major health policy focus. We analyzed demographic data of CML reported to ALN web portal patients presenting at 10 Medical Centers in Egypt. Better TKI adherence led to improved outcomes. ACAs adversely affected time and response rates to TKI and. ACAs were more frequent in younger, Imatinib resistant patients. For ACAs data refer to Second Report of Arab Leukemia Net Registry Part II.

References

[Part II of the Second Report of Arab Leukemia Net (ALN) Registry for Chronic Myeloid Leukaemia (CML) in the Middle East & North Africa Region (AFME). Additional Chromosomal Abnormalities (ACAs) in Egypt, a Multicenter Results]

Mohamed Azzazi1, Mervat Mattar2, Ashraf El Ghandour1, Manal El Sorady1, Samir Shehata1, Yasser El Nahassi1, Hani Hegabl1, Rashal brahimb1, Rasha Magdy1, Hend Ellithy2, Mohammad Shazly1, Rana Khaled2, Manal El Sorady1, Ashraf El Ghandour1, Mai Sameir1, Mohab Elshaer1
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Background
The first ALN report demonstrated that age-specific rates for CML in Egypt and Arab nations are lower by at least two decades compared to western populations. Lack of reliable data concerning geographic and ethnic variations and response rates to therapy in Arab nations contribute to the variability of incidences among CML registries. Studies underestimate the true age of CML patients depending on the ease of access to medical services that show great diversity in AFME region. ACAs were reported in 5% of CML patients in chronic phase (CP). At diagnosis considered by ELM as a “warning” careful patient monitoring, while ACAs emerging during treatment are considered by WHO classification as accelerated phase (AP). ACAs influence response to Imatinib and outcome of TKI therapy.

Objectives
1) To investigate the low mean age of CML in Egypt.
2) To evaluate role of ACAs in disease and clonal evolution.

Table 1: Comparison of patients’ characteristics according to ACAs response

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients with ACAs (N: 516)(89%)</th>
<th>Patients without ACAs (N: 578)(100%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, y</td>
<td>N 42/43 (range) 33 (24-64)</td>
<td>N 51 (18-84)</td>
</tr>
<tr>
<td>Sex, male/female, N 302/276 (%)</td>
<td>34/28 (86/14)</td>
<td>26/28/48 (69/41)</td>
</tr>
<tr>
<td>Median spleen, cm N (range)</td>
<td>9 (8-24)</td>
<td>6 (0-18)</td>
</tr>
<tr>
<td>Median mib level, grst N (range)</td>
<td>11.8 (8-21.0)</td>
<td>12.4 (6.4-17.3)</td>
</tr>
<tr>
<td>Median PLT count, 10^9/L (range)</td>
<td>408 (143-979)</td>
<td>346 (107-420)</td>
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<tr>
<td>Median peripheral blasts, % (range)</td>
<td>3.5 (1-8)</td>
<td>1.5 (0-10)</td>
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<tr>
<td>Median eosinophils, % (range)</td>
<td>3.9 (0-4)</td>
<td>2 (0-12)</td>
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<tr>
<td>Median basophil, % (range)</td>
<td>4.2 (0-13)</td>
<td>2.5 (0-12)</td>
</tr>
<tr>
<td>Phase of CML (%)</td>
<td>38/16%</td>
<td>46/30%</td>
</tr>
<tr>
<td>chronic phase N 503 (87.8%)</td>
<td></td>
<td>465 (90%)</td>
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<tr>
<td>Accelerated phase N 47 (8.1%)</td>
<td></td>
<td>33/6%</td>
</tr>
<tr>
<td>Blastic phase N 28 (4.9%)</td>
<td></td>
<td>18 (4%)</td>
</tr>
<tr>
<td>Sokal score, N 578 (100%)</td>
<td></td>
<td>314 (61%)</td>
</tr>
<tr>
<td>Low N (%) 334 (57.8%)</td>
<td>68 (32%)</td>
<td>314 (61%)</td>
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<tr>
<td>Intermediate N (%) 142 (24.9%)</td>
<td>77 (27%)</td>
<td>125 (24%)</td>
</tr>
<tr>
<td>High N (%) 102 (17.7%)</td>
<td>21 (4%)</td>
<td>77 (15%)</td>
</tr>
<tr>
<td>Hasford score, N 578 (100%)</td>
<td></td>
<td>318 (62%)</td>
</tr>
<tr>
<td>Low N (%) 341 (59%)</td>
<td>23 (37%)</td>
<td>318 (62%)</td>
</tr>
<tr>
<td>Intermediate N (%) 164 (28.4%)</td>
<td>31 (43%)</td>
<td>136 (25%)</td>
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<tr>
<td>High N (%) 73 (12.6%)</td>
<td>11 (18%)</td>
<td>62 (12%)</td>
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<tr>
<td>FUTOS score, N 578 (100%)</td>
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<td>18 (3%)</td>
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<tr>
<td>Low N % 339 (93%)</td>
<td>44 (11%)</td>
<td>46/96%</td>
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<tr>
<td>High N % 39 (7%)</td>
<td>18 (20%)</td>
<td>21 (4%)</td>
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<td>Variant Ph translocations, N (%)</td>
<td>4 (6%)</td>
<td>18 (3%)</td>
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<tr>
<td>Deletions del(9), N (%)</td>
<td>37 (2%)</td>
<td>47 (8%)</td>
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<tr>
<td>Imatinib dose (mg), N (%)</td>
<td></td>
<td>36 (7%)</td>
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<tr>
<td>400 N 400 (69%)</td>
<td></td>
<td>314 (61%)</td>
</tr>
<tr>
<td>800 N 178 (31%)</td>
<td></td>
<td>150 (25%)</td>
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</table>

8
Method
We analyzed data of 578 CML patients (followed-up for 5 years). Data collected according to ELN2 GIMEMA3 and EUTOS recommendations2.3 via a multicenter web portal, the ALN. (www. aln-affme.com). Chromosome banding analysis and FISH were performed according to the International System for Human Cytogenetic Nomenclature.

Results
Patients median age was 43y, Table 1. 98% of patients achieved CH response, 89% PCyR, 87% CCyR, and 83% MMR. ACAs were found in 62(11%) patients, they had lower cytogenetic and molecular response rates and longer response time to TKI and inferior outcome. ACAs were more frequent in younger, imatinib resistant patients, and in blast phase. We identified loss of Y chromosome in 18 patients (29%), trisomy 8 in 7 (11%), trisomy 19 in 12 (19%), tetrasomy 17q in 10 (16%), other single abnormalities in 8 patients (13%), complex karyotype in 5 patient (8%).Four patient showed variant Ph chromosome: t(9;22)(q34;q11), deletion of der(9) chromosome in 17 cases (27%), 10 cases with loss of Y, 4 case with del(20)(q11), and 3 case with del(3)(q21;q32). The cytogenetic and molecular response rates were uniformly lower in patients with ACAs, overall CCgR and MMR rates were significantly lower in patients with ACAs (68% versus 89% and 55% versus 86 respectively), responses were significantly slower in patients with ACAs. 54 patients presented with ACAs at diagnosis while 8 patients developed ACAs while on treatment.

Conclusions
The importance of ethnicity and gender differences in relation to disease incidence, and prognosis are major health policy focus. To investigate the low mean age of CML in Egypt and evaluate role of ACAs on disease and clonal evolution, we analyzed CML data of 10 Medical Centers in Egypt. We identified 62(11%) cases with clonal ACAs. ACAs are more frequent in younger patients and adversely affected time and response rates to imatinib treatment.

References

[6] Polymorphism of UGT1A1 and Frequency of Hyperbilirubinemia in Patients with Chronic Myeloid Leukemia Patients Treated by Nilotinib

A. Bykova, A. Abdullaev, G. Gusrarova, E. Chelysheva, S. Treglazova, A. Turkina
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Background
Isolated hyperbilirubinemia mostly of indirect bilirubin fraction is diagnosed in patients with polymorphism of UGT1A1 gene (Gilbert’s syndrome), mainly homozgyous genotype (TA7/T7) which encodes the enzyme uridinediphosphoglcoyxytransferase 1 (UDP-GT) in hepatocytes. Hyperbilirubinemia is also frequent laboratory abnormality in chronic myeloid (CML) patients treated by nilotinib. Connection of hyperbilirubinemia with UGT1A1 polymorphism in CML patients on nilotinib therapy requires understanding and studying.

Objectives
To estimate the correlation between polymorphism of UGT1A1 gene and frequency of hyperbilirubinemia in patients with CML treated by nilotinib.

Methods
We estimated biochemical parameters in a group of 100 patients treated by nilotinib: bilirubin, transaminases (AST, ALT), persistence of hyperbilirubinemia and biochemical parameters normalization. We also considered patients’ anamnesis for hepatitis and estimated those laboratory abnormalities in previous imatinib therapy as 82 of 84 patients received nilotinib second line after imatinib. Mean time of observation on nilotinib therapy was 36.7 months (range 1 - 94.5). Men/ women ratio was 45/55. Promoter region of the UGT1A1 gene was studied by allele specific polymerase chain reaction (AS-PCR).

Results
Hyperbilirubinemia due to the indirect bilirubin fraction was observed in 84 (84%) of 100 patients. Of those 84 patients hyperbilirubinemia grade 1 was in 41 (49%), grade 2 in 33 (39%), grade 3 in 10 (12%). Normal genotype (TA6/T6) was in 71 (71%) patients, heterozygous genotype (TA6/T7) in 19 (19%), homozygous genotype (TA7/T7) in 10 (10%) patients. Frequency of hyperbilirubinemia grade 1-3 in patients depending on genotype is presented in table1. Hyperbilirubinemia grade 1 was associated mostly with normal genotype patients, grade 2 in with normal and abnormal genotype, grade 3 with abnormal and homozygous genotype (9 of 10 patients). In 1 patient with normal genotype grade 3 hyperbilirubinemia was due to intracellular hemolysis approved by laboratory tests. One patient with heterozygous form (TA6/T7) and normal bilirubin was on nilotinib <1 month. Hyperbilirubinemia on previous imatinib treatment was in 29 (35.7%) of 82 patients with

<table>
<thead>
<tr>
<th>UGT1A1 genotype</th>
<th>Number of patients</th>
<th>Normal bilirubin n(%)</th>
<th>Bilirubin increased n(%)</th>
<th>Bilirubinemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Grade 1 n(%)</td>
<td>Grade 2 n(%)</td>
</tr>
<tr>
<td>TA6/TA6 (normal)</td>
<td>71</td>
<td>15 (21.1%)</td>
<td>56 (78.9%)</td>
<td>37 (66%)</td>
</tr>
<tr>
<td>TA6/TA7 (heterozygous)</td>
<td>19</td>
<td>1 (5.3%)</td>
<td>18 (94.7%)</td>
<td>4 (22.2%)</td>
</tr>
<tr>
<td>TA7/TA7 (homozygous)</td>
<td>10</td>
<td>0 (100%)</td>
<td>10 (100%)</td>
<td>0 (30%)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>16 (16%)</td>
<td>84 (84%)</td>
<td>41 (49%)</td>
</tr>
</tbody>
</table>

Table 1: Frequency of hyperbilirubinemia grade 1-3 in patients treated by nilotinib according to UGT1A1 genotype
second line nilotinib: grade 1 in 25 (86.2%) of 29 patients (homozygous genotype TA7/T7 in 5 of 25), grade 2 in 4 (13.8%) of 29 patients (all with homozygous genotype TA7/T7). Normal bilirubin levels were in 55/65 (84.6%) of 82 patients on previous imatinib therapy. One patient with TA7/T7 genotype received nilotinib as first line. Bilirubin levels normalized in 48 (57.2%) of 84 patients during 1 to 3 months and persisted in 36 (42.8%). In 8 (9.5%) of 84 patients transient ALT and AST elevation was observed: grade 1(1), grade 2 (5) grade 3-4(2); it was resolved and only isolated hyperbilirubinemia was observed later on. In 2 of 84 patients hepatitis C was diagnosed. No treatment discontinuation was done due to hyperbilirubinemia.

Conclusions
In CML patients on nilotinib treatment Grade 3 hyperbilirubinemia as well as previous history of hyperbilirubinemia any grade on imatinib can be a sign of homozygous genotype TA7/TA7. Lower grades of hyperbilirubinemia occur both in patients with normal and abnormal heterozygous genotype. Other reasons for hyperbilirubinemia (hemolysis, hepatitis) should be assessed. No connection of UGT1A1 polymorphism and transaminase (ALT, AST) elevation was established.

[7] Deep sequencing of the bcr-abl kinase domain reveals a high frequency of bcr-abl35-ins insertion/truncation mutation in chronic myeloid leukemia and Philadelphia positive acute lymphoblastic leukemia patients

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Objectives and background
Although BCR-ABL kinase domain (KD) mutations can frequently be identified in patients who develop resistance to tyrosine kinase inhibitors (TKIs) in Philadelphia-positive (Ph+) leukemias, other mechanisms may play a role. Small insertions and deletions within the BCR-ABL KD have occasionally been reported in chronic myeloid leukemia (CML) patients who failed TKI therapy and have been hypothesized to have a causative role in drug resistance. Some were in-frame insertions and deletions, others were predicted to result in truncated BCR-ABL proteins. However, the detection of these sequence variations is hampered by the fact that they are almost always confined to subclones co-existing with full length BCR-ABL. This has most likely resulted in an underestimation of their frequency and complexity, since i) they can be confused with background noise/reduced quality reads in direct sequencing chromatograms and ii) cloning would be needed to better resolve overlapping sequences in these samples. The recent development of Deep Sequencing (DS) technologies has opened the way to a more accurate characterization of molecular aberrations. DS enables greater sensitivity, quantitation of sequence variant abundance and clonal analysis of a given DNA region. We thus took advantage of 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 175 samples of 77 CML and 24 Ph+ ALL patients who received one or multiple lines of TKI therapy were analyzed. DS was performed on a Roche GS Junior instrument, according to an amplicon sequencing design and protocol set up and validated in the framework of the IRON-II international study. Runs were designed to achieve high sequencing depth; this allowed to reliably identify and characterize 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 56/77 (73%) CML and 21/24 (87%) Ph+ ALL patients. This sequence variation, already reported in the literature as ‘35INS’, consists in 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 a.a. encoded by intron 8 sequences but lacking a.a. 359-383 of the KD, including 22 a.a. of the KD, along with the entire C-terminal region. 35INS was detected with variable abundance (range 0.1%-97% of all BCR-ABL transcripts), but in only 12 samples abundance was higher than 15-20% - thus detectable also by conventional sequencing. Resequencing 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 26/77 (33%) CML patients and 14/24 (58%) 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, that 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. The 35INS was also detected in the ABL counterpart in healthy controls. Further suggesting that is probably just an aberrant splicing isoform devoid of any pathogenetic role. 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. However, further analysis of a larger number of samples would be needed to better understand the biological and clinical meaning of these minor clones surviving TKI therapy. Supported by ELN, AI1, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project.
Standard and High Sensitivity Molecular Analysis in Chronic Myeloid Leukemia Patients Candidate to Discontinuation of Tyrosine Kinase Inhibitors

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Objectives and background
The introduction of Tyrosine Kinase Inhibitors (TKIs) has dramatically changed the prognosis of chronic myeloid leukemia (CML). For patients who maintain deep molecular remission for many years, it is now under discussion the possibility to discontinue TKI therapy. Aims of our study were to identify a subset of patients who achieved deep and durable molecular remission under TKI therapy potentially suitable for future TKI withdrawal, and to assess the real depth of molecular response by Replicate-PCR, a higher sensitivity PCR in selected patients willing to discontinue TKI therapy.

Methods
We reviewed molecular data from all chronic phase CML patients (102) followed in our Center in order to identify patients showing either undetectable BCR-ABL transcript (at least MR4) or detectable transcript <0.0032% (MR 4.5) during the last two years (here defined as CMR). Minimal residual disease was also assessed with a new strategy of Replicate RQ-PCR by using a 82 well plate, each performing an amplification reaction, that demonstrated approximately a 2 log improvement in detection sensitivity limit compared to conventional RQ-PCR. Results from patient samples were compared with 12 normal controls, which resulted in positive amplification in 6 out of 98 total wells with a mean number of positive wells of 0.5 (SD 1.0).

Results
Among 102 patients we selected a 76 patients were diagnosed after year 2000 and treated with TKIs, with a minimum follow-up of 2 years. We found that 23 (30.2%) patients met the discontinuation criteria we previously described; out of 23 patients, mean time from start of last treatment to CMR was 25 months (3-94), median duration of CMR was 70 months (24-139). 15 patients (65.2%) were receiving Imatinib, 8 (34.8%) were receiving 2nd generation TKIs, in particular 5 nilotinib (4 as first-line treatment) (21.7%), 2 dasatinib (1 as first-line treatment) (8.7%) and 1 bosutinib (4.3%). Mean time to CMR was 29 mos (4-94) in patients under frontline Imatinib, and 13 mos (6-24) in patients under frontline nilotinib. Replicate-PCR was performed on 7 of these patients (including 2 patients off-treatment). Samples from 2 other patients already off-treatment who had started therapy before year 2000 were also analyzed.

Conclusions
In a single center experience, a significant percentage of patients treated with TKIs (around 30%) may eventually reach criteria for discontinuation. High sensitivity molecular analysis demonstrate that residual transcript is evident in some patients who remain in stable MR4.5, thus indicating that this population of patients is still heterogeneous. The role of Replicate-PCR deserve further evaluation.

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Clinical significance of the rate of BCR-ABL decline for patients with chronic myeloid leukemia in chronic phase on tyrosine kinase inhibitors treatment

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Objectives and background
About 70% CML patients on TKI treatment, with early molecular response (BCR-ABLIS <10% at 3-months), have 5-year overall survival of 95%. Nonetheless, CML patients remain heterogeneous group and several studies in recent years were aimed to personalize treatment based on individual patients’ characteristics. One of them was the study by B. Hanfstein et al. (2014), which showed good prognostic potential of 0.35 ratio BCR-ABL level at 3 months to absolute transcript level at diagnosis. In this study, GUS was used as control gene, but at present ABL is normalization gene for BCR-ABL quantification worldwide. The aim of our study was to assess potential of ratio BCR-ABL level at 3 months to baseline using ABL as control gene to predict optimal response.

Methods
Twenty-six patients (median age, 52 years; range 24-84; 11 male and 15 female) with chronic phase CML were included in the study, 4 patients had EUTOS high-risk. 16 patients started treatment with Imatinib 400 mg/day and 10 patients started with Nilotinib 600 mg/ day. Median BCR-ABLIS transcript levels was 36.883% at diagnosis, range 3.390-3185.361%. BCR-ABL levels were monitored at diagnosis and at 3, 6 and 12 months of treatment. The ratio of BCR-ABL levels at 3 months to baseline for each patient was calculated. We performed ROC curve analysis to establish the best cut-off value to predict MMR achievement as optimal treatment results at 12 months. Then we compared predictive sensitivity of our ratio cut-off and early molecular response at 3 months (10% by IS). Statistical analysis was conducted with ROC analysis and Fisher exact test.

Results
The ratio BCR-ABL at 3 months to baseline as 0.1 was revealed as best cut-off value to predict MMR at 1 year (fig. 1). 15 out of 17 patients (88.2%) with ratio below 0.1 achieved
MMR at 12 months, while only 2 of 9 patients (22.2%) with ratio more than 0.1 had optimal response (hazard ratio = 0.2625 (C.I. 0.027-2.56); p=0.0016). Using early molecular response at 3 months (10% by IS) yielded worse discrimination results: 17 of 23 (73.9%) patients with BCR-ABL <10% at 3 months had achieved MMR at 12 months, whereas none of 3 patients with BCR-ABL level >10% had MMR at 1 year (p=0.0323). Moreover application of our cut-off value among patients with BCR-ABL level less than 10% at 3 months allowed us to identify 4 high-risk patients have not reached MMR to a 1 year of therapy.

Conclusions
Our study demonstrated that the individual BCR-ABL decline rate from baseline to 3 months might be useful prognostic marker that allowed detecting more patients at risk who have no MMR at 1 year of treatment and ABL might be used as control gene.

References:

Objectives and background
Second-generation TKIs have demonstrated efficacy and an acceptable tolerability in patients (pts) with chronic myeloid leukemia (CML); however, new data from so called “off target” side effects have been published. For example, serious concerns have been raised about cardiovascular (CV) events with Ponatinib, and, in lesser degree with Nilotinib, impeding
or difficulting the treatment in patients with previous CV risk factors. Besides, patients with previous history of pleural effusion or pulmonary hypertension should avoid dasatinib (DA) if possible. Bosutinib could be a good candidate for situations which preclude the use of other TKIs. We have previously presented efficacy data of 29 patients treated with bosutinib in forth line. The aim of this study is to report safety data of heavily CML patients treated with bosutinib in 4th line.

Methods
We have studied 30 pts previously treated with imatinib (IM), dasatinib and nilotinib and 5 pts previously treated with IM-DA or NI since 2012 under the Spanish Compassionate Use Program. Patient’s baseline characteristics and previous treatments are shown in table 1. We have classified patients in 2 groups regarding to the reason why BOS was given: intolerant (INT) or resistant (RES). Toxicities were graded using the National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0. Event free survival was defined as previously described (Guilhot et al, Blood 2012).

Results
At the data cutoff on June 16, 2014, the median follow up was 11.47 months (range, 2.03-45.97 months). Median duration of BOS treatment across all cohorts was 9.23 months (range, 0.63-23.40 months). We observed no significant differences in terms of Index prognostic factors ( Sokal, Hasford or Euts), sex, median duration of TKIs treatment or comorbidities. However, patients with resistance where significantly older observed: 56 years vs. 67 years (p<0.05). Toxicity spectrum pre-BOS: Main reason for treatment discontinuation for each TKI was: treatment failure in the case of IM (14/35) and intolerance for both DA 16/34 and NI 13/31. Hematological (HEM) toxicities grade 3-4 with all TKIs were more common in RES pts, being dasatinib the one that showed the highest rate of grade 3-4 HEM toxicities. Non-HEM toxicities to all TKIs were significantly more frequent in INT than in RES pts (p>0.05). Most common grade 3-4 non-HEM toxicities were rash for IM (3/35), pleural effusion for DA (7/34) and vascular events for NI (3/31 Peripheral arterial disease (PAOD), 3/31 Ischemic heart disease (IHD). Toxicity spectrum with BOS treatment: treatment interruptions were more frequent in INT than in RES pts 52% vs 25%, as well as dose reductions 78% vs 66% respectively. Grade 3-4 HEM toxicities were more common in RES than INT pts (41.6% vs 4.3% respectively). Non-HEM toxicities were also more frequent in RES pts than INT: diarrhea (50% vs 43%), rash (16% vs 8%), ALT or AST increase (25% vs 13%) abdominal pain (16% vs 4%), grade 3-4 non HEM toxicities were more frequent in RES than INT pts (41% vs17%) (Diarrhea 16.7% vs 4.3%, AST/ALT increase: 16.7%vs 0%). None (0/12) vs 4/23 (17%) pts discontinued treatment due to toxicity in the RES vs. INT group respectively. Cross intolerance was extremely rare, of the 7 pts who had rash with IM, only 1 suffered rash with BOS. None pts had pleural effusion with BOS out of 15 who previously suffered with DA and 1/10 vascular events in pts that previously suffered with NI. EFS by 20 months was 75% vs 50% for INT and RES patients.

Conclusions
We have shown how in previously heavily pretreated CML patients, most of them in 4th line bosutinib has an excellent safety profile with no patients interrupting treatment due to side effects in previously intolerant patients. Importantly, rates of cross intolerance (namely: CV, pleural and skin ) have also been very low, We conclude that Bosutinib is an excellent alternative also in patients who are left without a suitable treatment option.

[11] Clinical significance of the rate of BCR-ABL decline (ratio of BCR-ABL level at 3 months to 1 month) for patients with chronic myeloid leukemia in chronic phase on tyrosine kinase inhibitors treatment

Mikhail Fominikh, Vasily Shuvaev, Irina Martynekevich, Dzhariyat Shikhbabeva, Alla Abdulkadyrova, Vera Udaleva, Regina Golovchenko, Irina Zotova, Lyubov Polushkina, Ekaterina Petrova, Lyudmila Martynenko, Elizaveta Kleina, Natalya Tsybakova, Kudrat Abdulkadyrov

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Objectives and background
Rapid reduction in BCR-ABL level is associated with a good therapy outcome. Currently the reduction of BCR-ABL level below 10% by 3 months of therapy is used as a criterion for the early molecular response. However, this level does not take into account the individual characteristics of the patient and the initial tumor volume. One of the obstacles to use of baseline BCR-ABL level is a distortion of the results of its measurement (non-linearity) due to the mixture of BCR-ABL with normal ABL control gene. During the first month of therapy there takes place a rapid tumor mass reduction. The aim of our study was to assess potential of ratio BCR-ABL level at 3 months to 1 month with using ABL as control gene to predict optimal response related to individual patient tumor characteristic.

Methods
Ten patients (median age, 55 years; range 24-84; 2 male and 8 female) with chronic phase CML were included in the study, three patients had EUTOS high-risk. One patient started

Figure 1: Proportion of patients in MMR and ratio of BCR-ABL level 3 month to 1 month
Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease characterized by t(9;22)(q34;q11), known as Philadelphia chromosome (Ph). This translocation creates oncogenic BCR-ABL fusion gene, which encodes the constitutively active BCR-ABL tyrosine kinase. Tyrosine kinase inhibitors (TKIs) designed to inhibit BCR-ABL activity have considerable improvement in all treated CML patients. Imatinib therapy led to achievement of complete cytogenetic response (CCyR) in majority of CML patients, and thus more sensitive measurements than cytogenetic analysis are necessary to follow up minimal residual disease. Real-time reverse transcriptase polymerase chain reaction (RQ-PCR) is the method of choice for detecting and quantification of BCR-ABL mRNA. RQ-PCR follow up in patients who are on long term imatinib treatment and in CCyR, enables additional information about the loss of optimal molecular response just on time. Accordingly there are further possibilities to change treatment, like dose escalation of imatinib or switching patient to second generation of TKIs.

Methods
Molecular monitoring was assessed by RQ-PCR according to EAC protocol [1, 2]. 79 CML patients, who were treated with imatinib for at least 3 years were followed up by RQ-PCR since 2012. All patients were diagnosed at the Clinic of Hematology, Clinical Center of Serbia. Assays were performed using the 7500 Real Time PCR System (Applied Biosystems). A standard curve was created using serial plasmid dilutions for BCR-ABL fusion, and ABL control genes (Ipsogene). The level of BCR-ABL/ABL was assessed according to published data [3] and patients were considered to be in molecular relapse when BCR-ABL/ABL>0.1%.

Results
The ratio BCR-ABL at 3 months to 1 month as 0.1 was revealed as best cut-off value to predict MMR at 1 year (fig. 1). Six patients with ratio below than 0.1 achieved MMR at 12 months in 83.3% (n=5), while patients with ratio more than 0.1 none have achieved optimal response (p=0.0238). Comparison group results: 74 of 100 patients (74%) with BCR-ABL level ≤10% at 3 months had achieved MMR at 12 months, whereas only in 6 of 85 patients (7.1%) with BCR-ABL level >10% were MMR at 1 year.

Conclusions
Our study demonstrated that the individual ratio of BCR-ABL level at 3 months to 1 month might be studied as more predictive landmark for change of TKI treatment even among these patients which have BCR-ABL, levels ≤10% at 3 months. Also ABL might be used as control gene to predict optimal response related to individual patient tumor characteristic.

[12] Molecular Monitoring Significance in Long Term Imatinib Treated Chronic Myeloid Leukemia Patients

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Objectives and background
Chronic myeloid leukemia (CML) is clonal myeloproliferative disease characterized by t(9;22)(q34;q11), known as Philadelphia chromosome (Ph). This translocation creates oncogenic BCR-ABL fusion gene, which encodes the constitutively active BCR-ABL tyrosine kinase. Tyrosine kinase inhibitors (TKIs) designed to inhibit BCR-ABL activity have considerable improvement in all treated CML patients. Imatinib therapy led to achievement of complete cytogenetic response (CCyR) in majority of CML patients, and thus more sensitive measurements than cytogenetic analysis are necessary to follow up minimal residual disease. Real-time reverse transcriptase polymerase chain reaction (RQ-PCR) is the method of choice for detecting and quantification of BCR-ABL mRNA. RQ-PCR follow up in patients who are on long term imatinib treatment and in CCyR, enables additional information about the loss of optimal molecular response just on time. Accordingly there are further possibilities to change treatment, like dose escalation of imatinib or switching patient to second generation of TKIs.

Methods
Molecular monitoring was assessed by RQ-PCR according to EAC protocol [1, 2]. 79 CML patients, who were treated with imatinib for at least 3 years were followed up by RQ-PCR since 2012. All patients were diagnosed at the Clinic of Hematology, Clinical Center of Serbia. Assays were performed using the 7500 Real Time PCR System (Applied Biosystems). A standard curve was created using serial plasmid dilutions for BCR-ABL fusion, and ABL control genes (Ipsogene). The level of BCR-ABL/ABL was assessed according to published data [3] and patients were considered to be in molecular relapse when BCR-ABL/ABL>0.1%.

Results
Optimal molecular response (ELN criteria, BCR-ABL/ABL ratio ≤0.1%) was present in 68 of 79 evaluated patients (86%); MR3.0 in 19 pts (24%), MR4.0 in 32 pts (40.5%) and MR4.5 in 17 pts (21.5%). Eleven patients (14%) had no optimal molecular response; 6 of them had BCR-ABL/ABL ratio >1% with confirmed loss of CCyR, while 5 patients had BCR-ABL/ABL ratio <1% without loss of CCyR. Three patients, who lost molecular and cytogenetic response, received nilotinib and their further controls showed better molecular response. One of them achieved MR5.0 after 15 months on nilotinib treatment, while two others achieved MR3.0 after 6 and 9 months on nilotinib. One patient lost molecular and cytogenetic response after discontinuation of imatinib therapy due to compliance and soon after being compliant he recovered previous response with standard dose of imatinib. Two patients lost both CCyR and MR and are currently started nilotinib treatment but it is early to evaluate their response. Five patients who didn’t lose CCyR continued with previous imatinib therapy and are frequently controlled.

Conclusions
Molecular monitoring of CML patients allows us to react quickly to the reappearance of pathologic BCR/ABL clones and to achieve again cytogenetic and molecular response with the other treatment options.

References:

in leukemic patients using ‘real-time’ quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) - a Europe against cancer program.
Background
Family planning is an important issue for chronic myeloid leukemia (CML) patients due to high efficiency of the therapy tyrosine kinase inhibitors (TKI), long-term survival of patients with a good quality of life. However, pregnancy cases are rare and rules for CML therapy during pregnancy are not established. It is a traditional to have many children in Uzbek families, so the demand for treatment strategy for such patients is specifically important. CML patients in Uzbekistan are treated within GIPAP program, without stable cytogenetic and molecular monitoring.

Objectives
To analyze data of pregnancy outcomes and CML therapy strategy during the pregnancy in women and in female partners of men treated with imatinib in Uzbekistan

Methods
We collected retrospectively and prospectively data of pregnancy cases in CML patients in Uzbekistan from 2005 to July 2014 including demographics, CML therapy, pregnancy outcomes, neonatal characteristics and observation of infants. Data structure and tasks were developed by Research Institute of Hematology and blood transfusion, Uzbekistan with collaboration Hematology Research Center, Moscow, Russia supported by The Max Foundation using GIPAP data.

Results
We revealed 52 cases of pregnancy in 43 CML patients treated by imatinib in Uzbekistan: 36 cases in female partners of 27 men and 16 cases in 16 women. Seven men became fathers for 2 times, 1 patient 3 times. 36 patients had chronic phase (CP) of CML, 7 had accelerated phase (AP) at the time of diagnosis. Pregnancy outcomes and therapy during pregnancy of 16 pregnancy cases in women were following:
1) 12 deliveries, 11 on time, 1 premature. Therapy tactics were following: without therapy (2), imatinib during 7 months of pregnancy (2), imatinib during whole pregnancy (7) hydroxyurea during the last month of pregnancy in newly diagnosed CML woman (1). Thirteen healthy children (including twins) with no congenital malformations and normal birth weight were born. Children were healthy during 1-year follow up.
2) One miscarriage happened in a woman who was without therapy at the time of conception till the termination of pregnancy.
3) Three pregnancies continue at present time. Treatment tactics in 2 women are: interruption of imatinib for the first trimester and the resumption of imatinib after 2 trimester terms, 1 patient continues imatinib during pregnancy.

Conclusions
We managed to collect and describe the cases of CML patient pregnancies in Uzbekistan. Conception of male patients on imatinib seems safe. A tendency of healthy children birth was revealed in women with CML on imatinib therapy. We observed no adverse pregnancy outcomes, with imatinib therapy throughout pregnancy. However, a small number of cases require further investigations.

[14] The Microanatomy of the Leukemic Stem Cell Niche in Chronic Myelogenous Leukemia

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Objectives and background
 Constituents of the bone marrow microenvironment (BMM) influence the proliferation, differentiation and location of hematopoietic stem and progenitor cells (HSPC). Dependent on their maturation stage, different subsets of HSPC are localized at distinct sites in the BMM. This location depends on HSPC-intrinsic, as well as HSPC-extrinsic factors (1). The BMM protects leukemic stem cells (LSC) from treatment with tyrosine kinase inhibitors (2) or chemotherapy (3). We, therefore, investigated the microanatomy of the LSC niche hypothesizing that it may differ from the normal HSPC niche.

Methods
We used a combination of confocal and 2-photon intravital microscopy (IVM) of the murine calvarium (1) and the well-described retroviral model of BCR-ABL1 chronic myelogenous leukemia (CML) and B-cell acute lymphoblastic leukemia (B-ALL).
Results
We show here that BCR-ABL1+ Lin−c-Kit+ Sca-1+ (LKS) CD150−CD48− (LKS SLAM) cells, which harbor the leukemic stem cell (LSC) fraction in this model, home to locations further away from the endosteum than their normal counterparts. Prior in-vitro treatment of BCR-ABL1+ LKS with imatinib mesylate, considered standard of care in CML, reverses this phenotype and the cells are found closer to the endosteum. Native BCR-ABL1, as well as the imatinib-resistant BCR-ABL1 point mutants BCR-ABL1Y253F, BCR-ABL1E255K, BCR-ABL1T315I and BCR-ABL1M351T have similar intrinsic catalytic activity, but the BCR-ABL1Y253F, BCR-ABL1E255K, and BCR-ABL1T315I mutants increase the IL-3 independent proliferative capacity of 32D cells relative to native BCR-ABL1. BCR-ABL1Y253F and BCR-ABL1M351T cause increased transformation of primary BM B-lymphoid progenitors in vitro and lead to accelerated induction of B-ALL in mice. In the CML model, BCR-ABL1T315I and BCR-ABL1M351T induce myeloproliferative neoplasia with shortened survival and features of accelerated phase disease compared to native BCR-ABL1, whereas BCR-ABL1T315I LKS cells home closer to osteoblastic cells than LKS cells expressing native BCR-ABL1. Sequential in vivo tracking of leukemic progenitor growth by IVM shows a similar nadir in the number of cells per leukemic cell ‘nest’ 11 days after irradiation and IV transplantation in recipients of DsRed2− BCR-ABL1+ or empty vector control-transduced bone marrow. However, between days 18–25 after transplantation there is a significant increase in the number of cells per leukemic cell ‘nest’ compared to the empty vector control group. Sequential immunohistochemistry and TUNEL assays of leukemic bone sections in imatinib-or vehicle-treated recipient mice with CML show that initial BCR-ABL1+ growth trends to occur at locations further away from the endosteum, whereas erythroid islands are found closer to the endostium and trabeculae. Apoptosis in response to imatinib appears most prominent in the metaphysis. Lastly, we could demonstrate by IVM in the CML model that treatment of mice with a combination of imatinib plus granulocyte colony-stimulating factor leads to ‘emptying’ of the LSC niche and superior eradication of BCR-ABL1+ leukemic cells compared to treatment with imatinib alone.

Conclusions
In summary, these data suggest that the microanatomy of the LSC niche in CML differs from the normal hematopoietic niche. Mutation status of CML LSC plays a role in positioning in the microenvironment, and location in the niche may be altered pharmacologically, suggesting that niche location may influence clinical outcome.

References


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Objectives and background
Great knowledge about efficacy of new tyrosine kinase inhibitors (TKI2) in treatment of patients with chronic myeloid leukemia (CML) was obtained. Information about TKIs for long-term toxicity is smaller. Inhibition of off-target tyrosine kinases, which functions are not completely understood, can lead to unpredictable consequences. According to these facts it seems interesting to study side effects of TKIs in treatment and evaluate their influence to patients’ life.

Methods
76 CML CP, Ph+, patients, ≥18 years were included. All patients received TKI2 therapy (nilotinib, dasatinib) after imatinib (IM) stopping. Median age at the TKI2 start was 49 years (26–75). Median duration of TKI2 treatment was 17.2 mos (1-103). IM therapy was stopped in 11.8% (9/76) due to intolerance, 72.3% (54/76) due to resistance, 15.9% (12/76) due to intolerance+resistance. The duration of previous IM treatment was 24.9 mos (1.7-127). The toxicity grade was assigned according to NCIC-CTC criteria.

Results
Therapy was modified or concomitant treatment prescribed due to TKI2’s toxicity in 52.6% (40/76) pts. Hematological toxicity (HT) grade 3-4 was revealed in 36.8% (28/76) pts: neutropenias – 11.8% (9/76), thrombocytopenias – 7.9% (6/76), bi- or pancytopenias – 17.1% (13/76). Isolated anemia grade 3-4 was not revealed. In the majority of patients – 64.2% (18/28) - HT was revealed or persisted after 3 mos from TKI2 start. Nonhematologic toxicity (NHT) gr. 2-4 was revealed in 28.9% (22/76) of patients, and the combination of HT and NHT in 14.5% (11/76). Laboratory abnormalities dominated among NHT: 4 cases of high enzymes level (amylose and/or lipase without clinic of pancreatitis), 4 cases of high bilirubin level and 4 cases of hepatitis. 2 patients had hyperglycemia during nilotinib treatment, only 1 need concomitant treatment. 4 patients with history of vascular diseases, developed life threatening conditions: myocardial infarction(n=1, nilotinib), ischemic stroke(n=1, nilotinib), paroxysmal heart rhythm disorders (n=2, dasatinib). 2 patients after more than 2 years of TKI2 treatment developed other malignances. Pleural effusion gr.1 was revealed in 3 patients after more than 2 years of dasatinib treatment. Only in 1 case it was severe and dasatinib was stopped. The combination of different NHT types was in 9.2% (7/76) patients. NHT also developed predominantly after 3 mos of treatment – 81.8% (18/22). Toxicity in 31.6% (24/76) lead to decrease of TKI2 dosage. Treatment interruptions were made in 40.8% (31/76), median interruption duration during the first 12 months was 30 days (1-140). TKI2 therapy was stopped in 48.7% (37/76) patients mainly due to resistance. Intolerance and combination of toxicity and resistance were the reason for TKI2 discontinuation in 10.8% (4/37) and 8.1% (3/37) cases, respectively. Complete cytogenetic response was achieved in 47.9% (36/77) of all patients and it was not much lower in patients with toxicities - 40% (16/40), p<0.1.

Conclusions
More than 50% of our patients had HT or NHT. The majority of toxicity cases were temporary and resolved after treatment interruption. The main reason for treatment discontinuation was resistance, due to toxicity TKI2 treatment was stopped only in 10% of patients. No fatal cases of toxicity were registered in our patients.
[16] FoxM1 post-translational modifications associated with the BCR-ABL1 fusion gene of chronic myeloid leukemia

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Objectives and background
Signals promoting proliferation and survival of BCR-ABL1+ leukemic stem cells (LSC) are subject to intense investigation. Our work was focused on FoxM1, a member of the Forkhead transcription factor family and a central component of b-catenin activation. Indeed, b-catenin is a key signal for BCR-ABL1+ LSC self-renewal and persistence under TK inhibitor therapy. As following its nuclear import, it binds the T-cell factor/lymphoid enhancer factor 1 (TCF/LEF1) to form a transcriptionally active complex which triggers critical genes for leukemic hematopoesis proliferative advantage1-4. FoxM1 activation in chronic myeloid leukemia (CML) may be contingent upon multiple BCR-ABL1 tyrosine kinase (TK)-associated events, encompassing Polo-like kinase 1 (Plk1), p53, E2F transcription factors and Cdh1 (a component of the anaphase-promoting complex/cyclosome [APC/C] involved in M/G1 progression). It might be therefore regarded as a putative target for the selective eradication of BCR-ABL1+ LSC.

Methods
The investigation was carried out in cell lines (K562 and 32D cell clones transduced with a temperature-sensitive mutant of BCR-ABL1), bone marrow cell from 15 CML patients at diagnosis and 4 CML patients who reached MMR (major molecular response) after TKs therapy. TK inhibitor imatinib and Plk1 inhibitor BI-2536 were used to define the signal transducing pathway leading to FoxM1 activation in BCR-ABL1+ cells. PCR amplification was performed to assess the expression of FoxM1 and Cyclin D1, a b-catenin downstream gene critical for BCR-ABL1+ cell proliferation. Western blot and Immunoprecipitation analyses were performed to study FoxM1, b-catenin, Cyclin D1 and Plk1 protein levels and phosphorylation in sub-cellular compartments (nucleus and cytoplasm).

Results
The experiments carried out in BCR-ABL1+ cell lines and bone marrow cell from CML patients showed that:
1) FoxM1 activation associated with BCR-ABL1 TK proceeds from post-transcriptional events encompassing the protein phosphorylation at serine-threonine residues and driving Plk1 activating phosphorylation;
2) phosphorylation is a key event for FoxM1 interaction with b-catenin and Plk1;
3) Plk1 inhibition, besides reducing the interaction of FoxM1 protein with b-catenin through events encompassing Plk1-induced phosphorylation of FoxM1, results in a significant reduction of FoxM1 transcript, suggesting a feedback mechanism in the regulation of FoxM1 expression in BCR-ABL1+ cell context.

Conclusions
BCR-ABL1 promotes FoxM1 post-translational modifications possibly involved in the b-catenin nuclear import and transcriptional activation. Accordingly, FoxM1 may be a putative drug target to implement the therapy of CML in LSC compartment.

Acknowledgments
Umberto Veronesi Foundation, ELN, BolognaAIL, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project are acknowledged for financial support.

References


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Objectives and background
Cell response to stress is a central component of genomic stability. It encompasses signals involved in cell cycle arrest, chromatin remodeling and DNA repair. In this context, Gadd45 proteins function as stress sensors and gene transcription regulators1. Their expression is controlled by epigenetic mechanisms affecting DNA methylation2. Genomic instability is one major trait of chronic myeloid leukemia (CML)3. It is driven by the constitutive tyrosine kinase (TK) activity of BCR-ABL1 fusion protein, which concurrently upraises the levels of endogenous DNA damage and reduces the proficiency of DNA repair hence promoting the outcome of additional genomic alterations, which mark the disease progression4. Gadd45a down-regulation is a component of genomic instability in CML. It is associated with BCR-ABL1 TK and also contingent upon the interaction with Aurora kinase A (Aka), a member of a serine-threonine kinase family active during mitosis5, which has been advanced as a target for CML therapy.

Methods
Experiments were conducted on K562 cell line treated with imatinib (IM) and with the demethylating agent 5-Aza-CdR, on mononuclear cell fraction from bone marrow samples of 6 CML patients at diagnosis and 4 CML patients who developed resistance to TK inhibitors not contingent upon BCR-ABL1 point mutations. PCR amplification was used to assess Gadd45a transcript level. Chromatin immunoprecipitation (ChIP) was used to analyze 5 methyl cytosine (5mC) content and DNMT1 recruitment at a Gadd45a promoter region critical for gene transcription.

Results
Gadd45a levels in K562 cells were significantly upraised in response to IM, supporting that the gene expression is deregulated by BCR-ABL1 TK. 5mC and DNMT1 content at Gadd45a promoter were concurrently reduced by 4th up to 24th h of IM treatment. A significant increment of Gadd45a transcript proceeding from the
demethylation of its promoter followed K562 treatment with 5-Aza-CdR since 24h up to 72h. The findings reinforce the role of the methylation status of Gadd45a promoter in the regulation of gene expression in BCR-ABL+ cells. Moreover, we found a significant reduction of Gadd45a expression in 6 IM-sensitive CML patients at diagnosis and in 4 CML patients who developed IM resistance compared to healthy donors. In both groups of patients, Gadd45a downmodulation was associated with promoter hypermethylation and enhanced DNMT1 recruitment.

Conclusions
Gadd45a transcriptional downregulation associated with BCR-ABL1 TK is driven by promoter hypermethylation mediated by DNMT1. However, the mentioned above events does not affect the outcome of IM resistance. Still, they might contribute to the disease clonal evolution towards the fully transformed phenotype of blast crisis, hence supporting the clinical use of demethylating agents to improve the disease prognosis.

Acknowledgments
Umberto Veronesi Foundation, ELN, BolognaAll, AIRC, PRIN, progetto Regione-Università 2010-12 (L. Bolondi), FP7 NGS-PTL project are acknowledged for financial support.

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[18] GABPα is positively correlated to BCR-ABL/ABL ratio and PRKD2 expression in human CML and influences imatinib sensitivity in vitro

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Objectives and background
Chronic myeloid leukemia (CML) is characterized by the reciprocal translocation t(9;22) leading to a constitutively active ABL kinase as disease driver. For tailored treatment, specific tyrosine kinase inhibitors (TKI) were developed. Still, drug resistance is frequently observed and typically caused by point mutations in the ABL kinase domain. However, additional mechanisms promoting disease progression and TKI resistance are discussed. The GA-binding protein (GABP) consists of two distinct subunits, GABPA and GABPB, and is an important regulator in myeloid and lymphoid differentiation in mice. Moreover, its involvement in murine CML-like myeloproliferative disease (MPD) was recently confirmed as a potential GABP target in human CML.

Methods
GABPA expression was measured by qPCR in 70 untreated CML patients at time of diagnosis. In vitro, we studied effects of GABP dosage and functional impairment in response to imatinib. Stable knockdown and ectopic overexpression of GABPA as well as overexpression of a dominant-negative acting GABPB TAD deletion mutant were established in the BCR-ABL-transfected hematopoietic cells led to prolonged survival of recipient mice. Focusing on these findings in murine models, we aimed to investigate GABP’s role in human CML.

Results
We detected a positive correlation of GABPA expression to BCR ABL/ABL ratio and PRKD2 mutant were established in the BCR-ABL+ cell lines K562 and NALM-1. Effects on cell proliferation and apoptosis were measured by WST-1, competition and Annexin V assays. Furthermore, we analyzed expression of the putative GABP target gene protein kinase D2 (PRKD2) in K562 as well as in primary CML applying qPCR and Western blotting.

Conclusions
In summary, our findings demonstrate that GABP plays a role in human CML pathogenesis, indicated by the positive correlation of GABPA to the BCR-ABL/ABL ratio, which is a rough estimate for the tumor cell burden in peripheral blood. In vitro, GABP had an effect on chemosensitivity to imatinib in K562 as well as in TKI-resistant NALM-1 cells. Moreover, PRKD2, described to affect MPD pathogenesis in mice, was confirmed as a potential GABP target in human CML. Further investigations are necessary to clarify how GABP is involved in BCR-ABL-driven transformation and whether GABP or its targets, e.g. PRKD2, may serve as a treatment option for TKI-resistant CML.

References
[19] CML: Results of 12-year-old therapy tyrosine kinase inhibitors in patients with late chronic phase chronic myeloid leukemia


Scientific Advisory Department for chemotherapy of myeloproliferative disorders, Federal State Budgetary Institution Hematology Research Center, Ministry of Healthcare of Russian Federation

**Objectives and background**
A significant amount of patients with chronic myeloid leukemia (CML) have started treatment by tyrosine kinase inhibitors (TKIs) in late chronic phase (CP) after IFN-α failure. The patients (pts) in late CP were treated by Imatinib (IM) and a substantial part of them has already been switched to second-generation tyrosine kinase inhibitors (TKI-2). However long-term results of therapy in late CP CML at long term follow-up (over 12 years) have not yet been presented. AIM To evaluate the long term results of TKI treatment including 12-years overall survival (OS), progression free survival (PFS), structure of mortality and current treatment of the pts with late CP CML.

**Methods**
In non-randomized, open-label study since July 2000- September 2001 have been included 79 pts with Ph-positive CML in CP resistant/ intolerant to IFN-α, who started therapy by IM. Male/female ratio 41:38, median (Me) age at diagnosis 39 (18-64) years. Me duration of CML since diagnosis 173 (13-310) month (mo). Me time of pretreatment by IFN-α was 26(0,5-156) mo. The status of one patient is unknown since July 2013.

**Results**
Me time of observation from the start of TKI treatment was 146,4 (3,6-162)mo. Me duration of IM therapy and TKI-2 therapy was 80 (2,4-155)mo and 57 (1,5-95)mo correspondingly. The 12-year OS was 68%, PFS was 66%. Cumulative incidence of complete cytogenetic response (CCyR) at TKI-1 (IM)+TKI-2 was in 64(81%) cases. The 12-year OS of pts with or without CCyR on 12mo of TKI therapy was 91% vs 48%, respectively. CCyR on IM therapy was achieved in 44(55%) pts; Me time to CCyR was 30 (4-91)mo. IM therapy continued in 23pts (29%), and 17 of 23 pts have stable major molecular response (MMR). TKI-2 received 34 pts. In 20pts (58% of 34pts) CCyR was for the first time achieved on TKI-2 as 2nd and 3rd line therapy (maximum term- 134mo including IM+TKI-2). Alive- 25 pts with TKI-2 therapy: 19 pts received TKI-2 as a 2nd line therapy (only 4 of 25 pts with MMR), 6 pts as a 3rd-4th line after the failure of >2 TKI-2. TKI therapy was discontinued in 8 pts: in 4pts with deep molecular response (bcr-abl<0.01%) it was stopped safely, 2 other patients stopped therapy by themselves with no proper monitoring and had progression and death. Two pts received hydroxyurea due to resistance to all available TKIs. Totally 24 pts died. Structure of mortality was the following: 1) progression of CML in 19pts; 2) comorbidities: cerebrovascular accident in 2 pts, intestinal tumor with liver metastases- in one case 3) 2pts- refusal from treatment.

**Conclusions**
TKI treatment is highly effective even in the group of late CML CP pts. Long-term results of TKIs therapy are described in a well and long tracked young socially active group of patients in late CP CML have shown that progression of CML remains a leading cause of death, with a frequency of 1.3% cases per year even in the era of the TKI-2.

[20] The Clinical Significance of the Early Molecular Response (MR) in CML Patients Treated by Imatinib (IM)

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**Objectives and background**
The level of early MR is important for the optimization of therapy and making a decision to a switch to 2nd line therapy in patients (pts) who have not achieved an optimal response (OR). According to the recent recommendations at definition of OR on CML therapy, pts must have the level of BCR-ABL transcript gene at 10% or less and Ph-positive cells 35% or less at 3 months. But, in half of all cases of pts with BCR-ABL >10% at 3 months progression events happen between 3 and 6 months. The goal of our research was to investigate the prognostic impact of a large BCR-ABL transcript amount at 3 months on the subsequent response and the long-term outcome of CML pts treated frontline with IM;

**Methods**
We have examined 185 pts, who have got IM from January 2010 up to the present. Molecular monitoring has been done regularly in all patients according to ELN recommendations. Median age was 49 years. All pts were in CP. BCR ABL transcript levels were assessed by real-time quantitative PCR.

**Results**
In our study 54% (100/185 cases) of pts achieved the optimal response with BCR-ABL transcript levels ≤10% at 3 months, 50.3% (93/185 cases) did it - with BCR-ABL transcript levels ≤1% at 6 months, and only 18% achieved the optimal response at 12 months. The comparative analysis has shown statistical differences in all characteristics in 2 groups of pts, who...
either achieved or not the optimal response at 3 months. Pts with BCR-ABL transcript levels ≤10% more often achieved CCgR at 6 months (p=0.0000), CqfR during all period (p=0.0004), MMR at 12 months (p=0.0000), MMR during all period (p=0.0012) and MR4 during all period (p=0.0000), pts had longer event-free (p=0.0432) and overall (p=0.0279) 4-year survival. In our center we have switched 6 patients to the 2nd TKI - those who didn’t achieve the optimal response at 3 months. The switching showed the positive influence on loss level expression of BCR-ABL gene in 5 out of 6 patients. After that all patients achieved the optimal response in the future. For example, we had one patient with failure of IM at 3 months. We switched him the therapy to NI in 5 months after the diagnosis. As a result the patient achieved CqfR at 1,5 months, and the deep molecular response 4,5 log at 3 months.

Conclusions

Early and deep responses to TKIs are predictive of long-term response and favorable survival outcomes. 3-month reduction in BCR-ABL transcript levels to >10% is a factor of bad effectiveness of TKI therapy and requires switching to the 2nd TKI. Timely switching to the 2nd TKIs allows us to achieve an optimal response in CML patients with level BCR-ABL >10% at 3 months.

References


[21] Potential of Digital PCR in CML Calibration

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Objectives and background

This study describes the first attempt to extend the definition of the BCR-ABL1 international scale (IS) with copy number (CN) ratios using digital droplet PCR (ddPCR). Digital droplet PCR allows for the determination of the absolute copy number of BCR-ABL1 as well as reference gene(s) in CML cDNA samples. A conversion factor (CFddPCR) between BCR-ABL1CN/ reference gene(s)CN and %IS may be calculated by combining ddPCR and BCR-ABL1IS. Such a CFddPCR would be static and once agreed upon would enable calibration of the BCR-ABL1 qPCR analysis e.g. using the certified ERM-AD623 BCR-ABL1 calibrator plasmid from the Institute of Reference Material and Measurement, Belgium.

Methods

Six EUTOS-standardised laboratories supplied cDNA from 14 to 20 BCR-ABL1 positive patient samples (n=100) and their locally obtained BCR-ABL1IS. Copy numbers of BCR-ABL1, ABL1, BCR and GUSB in the cDNA samples were measured and calculated in Vejle by ddPCR (duplicates) and by qPCR (triplicates). Samples with BCR-ABL1IS values exceeding 10 %, samples negative for BCR-ABL1 (due to cDNA dilution) or below 0.01 % and samples lacking a ddPCR or qPCR reference gene value due to technical issues were excluded, leaving 70 samples fulfilling the requirements for inclusion in the study. The BCR-ABL1IS range of the 70 samples was 1-10 %IS: 25 samples, 0.1-1 %IS: 35 samples and 0.01-0.1 %IS: 10 samples.

Results

From the ddPCR analysis (n=70), average reference gene ratios of ABL1:GUSB (1:2.0); ABL1:BCR (1:2.8) and GUSB:BCR (1:1.3) were obtained. Combining the copy numbers obtained by ddPCR of BCR-ABL1 and reference genes with the locally obtained BCR-ABL1IS values, tentative CFddPCR = 70 for BCR-ABL1/ ABL1, CFddPCR = 140 for BCR-ABL1/GUSB, CFddPCR = 200 for BCR-ABL1/BCR and CFddPCR = 170 for BCR-ABL1/GUSB^BCR (geometric mean) were calculated. The CFddPCR and the copy numbers calculated from the qPCR analysis performed in Vejle were used to calculate a BCR-ABL1IS for the 4 reference gene combinations of each sample. The concordance between the supplied local BCR-ABL1IS and BCR-ABL1IS calculated using the CFddPCR was determined as r2= 0.81 for BCR-ABL1/ABL1, r2= 0.61 for BCR-ABL1/GUSB, r2= 0.73 for BCR-ABL1/BCR and r2= 0.71 for BCR-ABL1/GUSB^BCR.

Conclusions

Digital droplet PCR was used to calculate BCR-ABL1IS conversion factors based on copy number ratios between BCR-ABL1 and the 3 WHO reference genes ABL1, GUSB, BCR and in addition the geometric mean of BCR and GUSB. Using these CF’s, a high degree of concordance to the BCR-ABL1IS obtained in 6 European EUTOS-standardised laboratories was obtained, taking into account the variability introduced from differences in reverse transcription efficiencies and the use of different reference gene combinations. Use of a CFddPCR could improve BCR-ABL1 analysis precision by enabling local IS calibration when appropriate e.g. when changing batches of critical reagents.
Objective and Background
Chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML) are the most common leukemias of the elderly (>43 years). However, the sequentional occurrence of CML followed by CLL in the same patient is extremely rare. CML, the most common myeloproliferative disorder, has a characteristic (t9:22) cytogenetic abnormality that involves fusion of the BCR gene on chromosome 22 with the ABL gene on chromosome 9. The BCR/ABL fusion results in constitutive activation of tyrosine kinase, which leads to uncontrolled proliferation of myeloid cells.

Case Report
A 52-year-old female referred to oncology clinic in January 2008 due to excessive sweating. Her performance status was good. The patient had no history of any disease, tobacco, ethanol, or illicit drug use. The initial blood chemistry tests were normal. But WBC count was 30000 count. µL (lymphocytes=54% and neutrophils=42%). Computed tomography (CT) scanning revealed bulky cervical lymphadenopathy (CL). One month later, flow cytometry analysis (e.g., liver function test (lactate dehydrogenase and creatine), lipids level, platelet, hemoglobin level) were normal. But WBC count was 30000 count. µL (lymphocytes=54% and neutrophils=42%).

Conclusion
We report a patient who developed CML 6 year after the diagnosis of CLL and after a few months, signs of CML were disappeared and she appeared CLL. This is first reported case of the appearance and disappearance of CML in patient with CLL.

Table 1: Laboratory Data

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<thead>
<tr>
<th>Variable(x)</th>
<th>Value</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (count.µL -1)</td>
<td>13000 – 30000</td>
<td>4000 – 9000</td>
</tr>
<tr>
<td>Hemoglobin (g.dL -1)</td>
<td>12.0 – 15.2</td>
<td>119 – 229</td>
</tr>
<tr>
<td>Platelet (count.µL -1)</td>
<td>153000 – 31000</td>
<td>117 – 329 (+ 103)</td>
</tr>
<tr>
<td>Lactate dehydrogenase (IU.L -1)</td>
<td>357 ≤ 639</td>
<td>119 – 229</td>
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[23] Baseline Quality of Life in Chronic Phase Chronic Myeloid Leukemia Patients with Resistance or Intolerance to Imatinib and its Potential Prognostic Value

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Objectives and background
The independent prognostic value of baseline quality of life (QoL) was reported for different types of cancer. We aimed to examine QoL in chronic phase chronic myeloid leukemia (CP CML) patients before second-line therapy and to analyze its prognostic value.

Methods
75 CP CML patients resistant or -intolerant to imatinib were enrolled in the prospective, multicenter, non-interventional study (mean age – 51.3 years old, SD 15.4; range – 22-83 years; male/female – 37/38). All the patients received dasatinib as the second-line therapy.
Background and objectives
Chronic myeloid leukemia (CML) is a myeloproliferative disorder that inevitably evolves to acute leukemia in the absence of real curative treatment and whose support has been turned upside down since the advent of targeted therapy with tyrosine kinase inhibitors.

Methods
We report the cases of 73 patients with newly diagnosed CML treated with tyrosine kinase inhibitors of 1st and 2nd generation between January 2003 and July 2012 in the department of clinical hematology of Farhat Hached hospital. The average age was 44 years with a sex ratio (M/F) of 0.92.

Results
Splenomegaly was the predominant clinical sign associated with major leukocytosis with an average of 154,635 elts/mm³. All patients were in chronic phase at diagnosis except one who was in accelerated phase. The Philadelphia chromosome was identified in 100% of cases with equivalent molecular transcript bcr-abl type b3a2 and b2a2. Imatinib mesylate was prescribed at the dose of 400 mg / day for all patients allowing to obtain complete hematologic remission in 96% of cases, a complete cytogenetic remission at 12 months in 48% of cases and major molecular response 18 months in 25% of cases. Dose escalation to 600 mg / day and 800 mg / day was indicated to respectively 16 and 7 patients. The treatment was well tolerated in the majority of cases. The use of second generation tyrosine kinase inhibitors was necessary for 30 patients to overcome imatinib resistance in 33.3% of cases, relapse in 20% of cases, intolerance in 6.6%, a progression of the disease in 23.3% of cases and suboptimal response in 16.8% of cases.

Conclusions
Thus, baseline QoL in CP CML patients resistant or intolerable to imatinib may be of prognostic value of second-line treatment outcomes.

References

[24] Chronic myeloid leukemia treated with tyrosine kinase inhibitors: About 73 patients
Regaieg H, Achour B, Bouslama E, Romdhani M, Ben Youssef Y, Khelif A.
Department of Clinical Hematology Farhat Hached University Hospital-Sousse-Tunisia
[25] Pharmacoeconomic simulation of CML treatment – significant factors in cost-utility analysis

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Objectives and background
The second-generation tyrosine kinase inhibitors (TKI2) in first-line treatment of chronic myelogenous leukaemia (CML) patients showed their advantages over Imatinib in respect of tolerability, rate and promptness of deep molecular responses obtaining. Nevertheless, both Nilotinib and Dasatinib has some considerations about long-term toxicities and higher price than Imatinib. One of the modern hypotheses for further improvement CML care is that TKI2 can give possibility to get long treatment free remissions (“cure”) for more proportion of CML patients than Imatinib. This advantage can ameliorate high TKI2 cost, especially on long-term perspective from gain of quantity (survival) and quality of life. Pharmacoeconomic modelling of CML treatment can establish limits when various strategies will become cost saving. The aim of our study was to conduct sensitivity analysis of Nilotinib and Imatinib first line pharmacoeconomic models in CML treatment and to assess proportion of incremental cost-utility ratio Nilotinib over Imatinib below than Willingness-to-pay limit.

Methods
We have used previously constructed Markov chain models1 to compare CML first-line treatment strategies with Imatinib or Nilotinib with subsequent therapy cessation in cases of complete molecular response 4.0 log (CMR4.0). We have chosen the model population size of 800 newly diagnosed CML patients in Russian Federation annually. 20-years’ time horizon was used. We have calculated total cost for the next twenty-year period (2014-2033) including the expenses for existing and newly diagnosed CML patients in population level. We have recalculated the total cost in Euros to make our results more representative. Simulation model was used for statistical analysis. We conducted multiple regression analysis to identify statistically significant factors for cost-utility ratio in each model; also, we did it for incremental cost-utility ratio (ICUR) per quality-adjusted life year (QALY) for Nilotinib over Imatinib. We have used Willingness-to-pay limit (WPL) as 25 500 € per QALY to assess proportion of ICUR below WPL.

Results
Statistically significant parameters in multivariate analysis for Nilotinib first-line model were: successful Dasatinib second-line treatment cessation rate, quantity of newly diagnosed CML patients per year and complete blood count analysis (CBC) cost. Multiple regression in Imatinib first-line model showed statistically significant factors as follows: successful Dasatinib third-line treatment cessation rate, quantity of newly diagnosed CML patients per year, CBC and Hydroxyurea cost. Statistically significant factors for pharmacoeconomic comparison Nilotinib and Imatinib in first line were: Nilotinib 600 mg drug cost, adverse events diagnosis and treatment cost during first year of treatment, quality of life utility in patients on high-dose Imatinib. Simulation results with original Imatinib price showed that ICUR would be less than WPL in 56.4% of cases (fig.1). Substitution of the cheapest Imatinib generic price demonstrated that Nilotinib strategy would be preferable (ICUR<WPL) only in 32.9% of cases.

Conclusions
Sensitivity analyses showed stability of both models. The results of simulation with different parameters substitution could be useful tool in decision-making process for healthcare authorities.

Reference
[26] Clinical efficacy of dasatinib in patients with chronic myeloid leukemia and resistance or intolerance of imatinib treatment

Moscow regional clinical research Institute named of M.F. Vladimirsksiy, Moscow

Objectives and background
Finding ways to overcome resistance to imatinib treatment in patients with chronic myelogenous leukemia (CML) led to the creation of two new drugs - II generation tyrosine kinase inhibitors - nilotinib (Tasigna) and dasatinib (Sprycel). High antitumor efficacy is proved by the results of clinical studies. The aim of our study was to investigate the efficacy of therapy with dasatinib in CML patients resistant or intolerant to imatinib treatment.

Methods
We studied efficacy of dasatinib treatment in group of 30 CML patients. Age distribution was from 25 to 75 years (Me=49-year). Group consisted of 12 men and 18 women. 22 patients were in the chronic phase CML, 8 - in the accelerated phase. Hydroa and/or interferon-alpha have been used for averaged 36 months (from 3 to 96 months). Before starting of hematological relapse patients received imatinib from 3 to 81 years. Before discontinuation was in 7 patients with long lasting MR4 due to knowledge of safe cessation (4), for conception (3). In 6 of 7

Results
Complete hematologic response (CHR) on dasatinib treatment obtained in all group of 30 patients. 24 patients reached CHR at 1 month of treatment, and other 6 patients reached CHO only at 6 month. Relapse-free for over 12 months were detected in 25 patients. Cytogenetic response obtained in 22 patients; however, one patient has failed to achieve CCR, although CHR remained. The median time to achieve the CCR was 12 months (from 6 to 24). Minimal cytogenetic response (MinCR) in two patients was obtained at 6 months of therapy. Molecular analysis revealed that major molecular response (MMR) was achieved in 11 patients in the range from 12 to 30 months. All this patients remained the CCO. Achieving and durable maintaining of MMR can be considered as the prognostic factor of treatment termination due to CML cure. The study shown that dasatinib has good tolerability profile. There were no registered adverse events associated with fluid retention (superficial edema, pleural effusions, pericarditis, ascites) in both dosing schema groups (100 mg OD and 140 BID).

Conclusions
Thus, our study has shown that dasatinib used in CML patients with resistant or intolerance of imatinib can be considered as the treatment opportunity with high antitumor effect and low toxicity.

[27] Treatment Cessation In Chronic Myeloid Leukemia Patients as a Part of Treatment Process: Toxicity Determined and Active Stop of Tyrosine Kinase Inhibitors

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Background
Clinical trials demonstrate a safe discontinuation possibility of tyrosine kinase inhibitors (TKI) in patients with deep molecular response (MR). However reasons and indications for TKI cessation as a part of treatment process have not been studied.

Objectives
To evaluate the eligibility of long term TKI cessation in CML patients with deep and long lasting MR. To describe the reasons of stopping therapy, principles and terms of observation without treatment, preserving and restoring of MR.

Methods
We summarized retrospectively and prospectively 25 TKI discontinuation cases in 2 clinics of Russian Federation (Moscow, St.Petersburg. 2008-2014). Inclusion criteria were: 1) Ph+ CML 2) MR4 (BCR-ABL<0.01%) for >12 months confirmed by >2 consecutive analyses 3) discontinuation of TKI treatment. Chronic phase (CP)/accelerated phase (AP) at diagnosis was 24/1. Sokal score low:intermediate:high was 15/8/1. Therapy before discontinuation was: imatinib 1st line (n=16), 2nd generation TKI (TKI2) 2nd-3rd line (n=9): 5 dasatinib/4 nilotinib. Me TKI duration was 7,2 (range 2,5-13) years, Me MR4 duration was 50 (range 12-97) months. IFN before TKI was received by 13(52%) of 25 patients for Me 18 months (2-60 months).

Results
We specify 2 reasons of TKI discontinuation: 1) adverse events (AE) of TKI (Toxicity Group), n=18 2) self decision of patient (Active Group), n=7 (Table 1).

In Toxicity Group therapy was stopped for 1) AE grade 1-2 in 5 of 18 patients: unstable angina (2), hepatotoxicity (1), acute renal failure (1), menstrual dysfunction and infections (1); 2) AE grade 1-2 in 13 of 18 patients including recurrent or long lasting: fatigue, edema, arthralgia, muscle cramps, diarrhea, recurrent pleural effusion. The key clinical decision was not to restart TKI after AE termination and to continue monitoring of BCR-ABL transcript levels by RQ-PCR. For Active Group self-made discontinuation was in 7 patients with long lasting MR4 due to knowledge of safe discontinuation (4), for conception (3). In 6 of 7
patients BCR-ABL monitoring was performed, 1 patient refused from monitoring. Treatment was restarted at major molecular response (MMR) loss (BCR-ABL>0,1%) or by decision of physician. In case of severe AE the patients were switched to alternative TKI. Thirteen (52%) of 25 patients were observed without treatment for Me 23 months (6-77 months), in 12 MMR was maintained, 1 patient being off treatment for 54 months refused from monitoring (Active Group). Therapy was resumed in 10 patients with MMR loss and in 2 without MMR loss, by physician decision. All MMR loss cases occurred within first 6 months. No CML progression observed. Response restoration to MR4 was in 8 of 10 patients in 3-16 months, in other 2 it is early to estimate.

Conclusions
Observation without therapy may become an option in CML patients with stable MR4 and recurrent/severe TKI toxicity. Self-declared decisions of young patients with durable deep MR should be accompanied by regular RQ-PCR especially within first 6 months after TKI discontinuation. Resuming therapy at MMR loss shows to be safe.

Table 1: Characteristics of CML patients according to reason of stopping TKI (n=25)

<table>
<thead>
<tr>
<th>Toxity Group</th>
<th>Active Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Me of age, years (min-max)</td>
<td>55 (27-74)</td>
</tr>
<tr>
<td>Ratio male/female (m:f)</td>
<td>9m:9f</td>
</tr>
<tr>
<td>Me duration of TKI therapy, years (min-max)</td>
<td>5,9 (2,5-13)</td>
</tr>
<tr>
<td>Me duration of MO4 at treatment cessation, months (min-max)</td>
<td>41 (12-97)</td>
</tr>
</tbody>
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[28] Case of inv(3)(q21q26) detection in Ph-negative bone marrow cells of chronic myeloid leukemia patient who developed myelodysplastic syndrome with transformation to acute myeloid leukemia

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Objectives and background
Chronic myeloid leukemia (CML) is a clonal hematologic disorder characterized by cytogenetic marker - translocation t(9;22) (q34;q11) resulting in Philadelphia (Ph) chromosome and bcr/abl fusion gene. The use of targeted therapies with selective tyrosine kinase inhibitor imatinib mesylate (IM) has improved significantly the results of CML treatment. Unexpectedly, clonal cytogenetic abnormalities (CCA) were noted in Ph-negative bone marrow (BM) cells from some patients who had a cytogenetic response to IM. We describe a IM-treated CML patient who had inv(3)(q21q26) in Ph-negative BM cells and developed myelodysplastic syndrome (MDS) transformed to acute myeloid leukemia (AML).

Methods
We used conventional cytogenetic BM cells analysis and fluorescent in situ hybridization (FISH).

Results
A 45-year-old man was diagnosed with accelerated CML phase in July 2004. BM cell
karyotyping revealed t(9;22)(q34;q11). IM treatment (600 mg) was started since February 2007. He achieved complete cytogenetic response in February 2008. FISH-analysis with DNA probe for bcr/abl rearrangement showed negative results. In September, 2010 (2.5 years since IM treatment started) karyotyping revealed inv(3)(q21q26). This time and later for all observation period t(9;22)(q34;q11) hasn’t been shown in patient’s BM cells both by karyotyping and FISH. BM cells karyotype is presented in fig.1. The inv(3)(q21q26) detection was associated with MDS features: originally with resistant leukaemia and then with severe anaemia and thrombocytopenia, these symptoms progressed for next 3.5 years under inv(3) (q21q26) persistence in patient’s BM cells. Blast cells count increased gradually in myelogram achieving 11.5%. In February, 2014, three years since inv(3)(q21q26) primary identification, morphological substrate of AML with numerous CD34-positive blast cells was revealed in BM trephine biopsy. Taking into consideration poor prognosis and the absence of HLA-compatible related donors the recommendation to make BM stem cells transplantation from unrelated donor was given.

Conclusions
The clinical implications of CCA in Ph-negative BM cells in IM-treated CML patients are still unclear. Rare cases have developed MDS or AML [1]. Now 23 cases of MDS or AML in CML patients with CCA in Ph-negative BM cells have been reported [1,2]. There is no observations of CML patients with inv(3)(q21q26) in Ph-negative BM cells, but Bumm et al. [3] describe a CML patient developed MDS associated with t(3;21) (q27;22). Both in this case and in our patient 3q26/EVI1 region is rearranged.

References

[29] Intermittent Imatinib Therapy in Patients with Chronic Myeloid Leukemia is the Real Basis of Routine Clinical Practice.

Region Clinical Research Institute named after M. Vladimirskiy, Moscow, Russia

Objectives
To evaluate efficacy of imatinib in correlation with median daily dose (MDD) in non-selected patients with CML receiving imatinib in routine practice.

Methods
Analysis has been done in non-selected group of 44 patients with CML in early chronic phase (ECP) who were receiving imatinib in routine practice. The recommended dose of imatinib at the onset of treatment was 400 mg daily. But MDD of imatinib, calculated as actual number of capsules taken reported by patients for the first 6 months of treatment was lower than the recommended dose and was 317.8 mg. We have estimated cytogenetic responses obtained every 6 months and progression-free survival (PFS) and overall survival (OS) at 5 and 8 years, defined by standard statistical methods.

Results
Group of 44 patients in ECP was divided into two subgroups depending on actual MDD (317.8 mg). In the first subgroup 30 of 44 patients MDD was higher than 317.8 mg and was 372.5 mg daily. In these patients we have seen major cytogenetic response (MCR) in majority of cases, 22 of 30 (73.3%): 9 patients (30%) reached complete cytogenetic response (CCR), 13 patients (43.3%) - partial cytogenetic response (PCR). 6 patients (20%) achieved minimal cytogenetic response (MCR). Only 2 of 30 patients (6.7%) didn’t reach cytogenetic response. In the second subgroup 14 of 44 patients MDD was lower than 317.8 mg and was 200.5 mg daily. In this subgroup MCR was registered in 35.7% of cases and there were no cytogenetic response in 64.3% of cases. 41 (93.2%) of 44 patients were alive by 5 years of therapy. Progression of CML was not the cause of death in 3 patients (6.8%). Thus, the 5-year OS was equal PFS and amounted to 93.2%. 35 patients (79.5%) were alive after 8 years of therapy. 9 (20.5%) of 44 patients died, 5 of them (11.4%) - due to disease progression (3 patients - to the acceleration phase, 2 - blast crisis), 4 - because of other reasons not related to CML. Thus, the 8-year OS was equal PFS and amounted to 79.5%. We have analyzed 5-years and 8-years OS in the second subgroup of 14 patients. All 14 (100%) patients were alive by 5 years, 4 (28.6%) of 14 patients died after 8 years, 3 of them (21.4%) - due to the progression of CML. Thus, the 5-year OS with a 50% deficient imatinib dose was 100%, 8-year OS - 71.4%.

Conclusions
Our investigation has shown high efficacy of imatinib in the treatment of CML largely based on the evaluation of remote results, despite the occasional arising treatment interruptions.

References
[30] The PI3K inhibitors BKM120 and RAD001 are effective against JAK2V617F mutated cells and synergize with ruxolitinib in in-vitro and in-vivo preclinical models

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Objectives and background
Janus kinase 2 mutation (JAK2V617F) represents the molecular alteration of the majority of chronic myeloproliferative neoplasms (MPN) emphasizing the central role of dysregulated JAK2 signaling. Enhanced activation of other downstream pathways including the PI3K/mTOR pathway has been documented as well. As we previously reported1, targeting mTOR by the RAD001 inhibitor resulted in inhibition of JAK2V6F mutated cells and produced clinical benefits in a phase I/II trial2. In this study we evaluated the effects of BKM120, a PI3K inhibitor, alone and in combination with mTORC1 (RAD001) and JAK2 (ruxolitinib) inhibitor in in-vitro and in-vivo MPN models.

Methods
BaF3 and BaF3-EPO murine cells expressing wild type (WT) or JAK2 or human V617F mutated HEL and SET2 cell lines and primary MPN CD34+ cells from patients with MF or polycythemia vera (PV) were used to evaluate cell proliferation, colony formation, apoptosis, cell cycle and protein phosphorylation status. Effect of drug combination was analyzed according to Chou and Talalay calculating the combination index (CI); a CI <1 indicates synergistic activity. In-vivo studies were performed with the JAK2 VF Knock-In mouse model generated in C57B16/J strain by insertion of the reversed JAK2V617F exon 13 sequence; mating with Vav-Cre transgenic mice activates the V6F allele producing a MPN phenotype in progenies with V6F heterozygous expression3. Blood, spleen and bone marrow cells were analyzed after 15 days of treatment.

Results
We found that BKM120 preferential inhibited BaF3 and BaF3-EpoR VF cells (IC50: 364±200nM and 1100±207nM, respectively) and induced apoptosis (IC50, SET2=10µM, BaF3-EpoR VF=1.8µM). Western blot analysis showed marked reduction of phospho-mTOR and its target phospho-4EBP1 as well as downregulation of phospho-STAT5 at 6 and 24h of treatment. BKM120 impaired colony formation from MF and PV CD34+ cells at doses 2 to 8-fold lower than healthy controls (p<.01). BKM120 strongly inhibited EEC colony growth from PV pts (IC50, 9=4mM). Triple combinations including BKM120/ruxo plus either RAD001 (Torc1 inhibitor) or PP242 (Torc1/2 inhibitor) resulted highly synergistic (median CI=0.27 and 0.52) to indicate the importance of complete mTOR inhibition. Co-treatment of KI mice with 30mpk BKM120 + 30mpk ruxo + 1.5mpk RAD001 resulted in reduction of leucocytosis, reticulocyte count and improvement of splenomegaly: median spleen index (S.I.), calculated as spleen and body weights ratio per 100, was 1.1 in triple-treated cohort versus 2, 2.3, 3.4 and 3.6 in 60mpk BKM120, 60 ruxo, 3 RAD001 and Vehicle respectively. The level of phospho-STAT5 and -4EBP1 in the spleen was significantly reduced in mice receiving triple-treatment as compared to single drug treatment.

Conclusions
Complete inhibition of PI3K pathway associated with JAK2 inhibition resulted in marked antitumor activity in preclinical models of JAK2V617F mutated MPN models and provide a rationale for future combination clinical trials.

References
3. Hasan et al, Blood 2013;122(8)

[31] CALR Mutation Analysis through High Resolution Melting in Essential Thrombocythemia and Persistent Thrombocytosis. Clinical Associations

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Objectives and background
Around 50-60% of patients with primary myelofibrosis (PMF) and essential thrombocythemia (ET) carry JAK2 mutations and 5-10% present mutations in MPL. Recently, mutations in calreticulin gene (CALR) have been described in JAK/MPL-negative cases of ET and PMF, and its clinical, cytogenetic and molecular implications are a challenge. We studied the presence of CALR, JAK and MPL mutations in a series of patients with ET and persistent thrombocythemia suggestive of MPN (PT-MPN). We tested the feasibility of high-resolution melting (HRM) as a screening method for rapid detection of CALR mutations. We aimed to compare clinical, demographic and molecular features of CALR+ vs. JAK2/MPL+ subjects.

Methods
This retrospective study included 81 ET and 45 PT-MPN. The diagnosis of ET was according to the World Health Organization (WHO), and those cases that did not accomplish all WHO criteria for ET were classified as PT-MPN. CALR mutation analysis was performed through HRM. Sensitivity and specificity of HRM was tested by using serial dilutions of a mutant and by analyzing a series of 45 wild-type samples. The same HRM product was bidirectionally sequenced when curves diverged from wild-type. For statistical analyses SPSS statistical package (v. 15.0) was used.
Results

HRM was reproducible, with no false positive or negative and the limit of detection was of 3%. Among ET patients, 54.3% (44/81) were JAK2V617+, 1.2% (1/81) MPL+ and 21% (17/81) CALR+, of which, 9 (52.9%) were 52 bp deletions (L367fs*46), 7 (41.2%) 5 bp TTGTC insertions (K385fs*47) and other was a complex mutation. The incidence of CALR mutations in ET JAK2V617-negative was of 46% (17/37). Among patients with PT-MPN, 44.4% (20/45) were JAK2V617+, 2.2% (1/45) MPL+ and 8.9% (4/45) CALR+, among them, 2 were L367fs*46 and other 2 were K385fs*47, one of them, although clearly identified, evidenced low mutant allele burden. CALR polymorphism, rs148604781, was identified in one patient. The two main types of CALR mutants were distinguishable through HRM since they displayed characteristic difference plot patterns. The mean hemoglobin level was 14.9 g/l higher in JAK2+ (145.8 g/l) compared to CALR+ (130.9 g/l) patients (95% confidence interval, CI, 6.23g/L, p=0.02). The median platelet count was significantly higher in CALR+ (967.5x109/L) versus JAK2+ (701.0x109/L) patients (p<0.001). Patients carrying JAK2 mutations presented lower leukocyte count than those with CALR mutations (median 9.77x109/L vs. 7.97x109/L, p=0.025). JAK2+ patients were at higher bleeding and thrombotic risk with a relative risk of 1.6 (95% CI, 1.2-7, p=0.027).

Conclusions

- HRM is a closed tube fast screening method so sequencing is just performed in positive samples. Sensitivity of HRM is higher than Sanger’s therefore is a more accurate technique for MPN diagnosis.
- The incidence of CALR mutations in ET, 46% of JAK2-negative patients, was lower than reported.
- CALR mutations were associated with higher platelet count and JAK2 mutants with higher hemoglobin levels and leukocyte count.
- JAK2+ patients were at higher bleeding and thrombotic risk compared to CALR.

[32] A Comprehensive Algorithm and Methodology for the Diagnosis and Monitoring of Myeloproliferative Neoplasms with Calreticulin Mutations

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Objectives and background

Somatic mutations in exon 9 of the Calreticulin (CALR) gene have been identified in the majority of JAK2 wild-type MPN. The discovery of CALR mutations, and the mutual exclusivity between mutant CALR, JAK2 and MPL genes have increased the proportion of MPN patients with a molecular marker extending the applicability of genetic diagnosis to over 90% of all MPN patients. Many clinical laboratories detect CALR exon 9 mutations using sequencing, a time-consuming and costly method, while the demand for high-throughput testing is increasing. In this context, an urgent need arises for reliable clinical laboratory diagnostic tests and a diagnostic algorithm for the detection of these mutations.

Methods

A Real-time PCR method was set up with a common reverse primer and a single probe for wild type DNA, and the two most common mutations, to simplify the assay. High resolution melting (HRM) analysis was standardized, with primers covering the region with all frameshift mutations published so far.

Results

We reviewed all published CALR mutations that have been identified in patient samples, and the resulting alterations in the amino acid sequence for each one of the novel coded proteins 1-3. To date, 55 individual CALR mutations have been described with the two most common variants being a 52 bp deletion (type-1) and a 5 bp insertion (type-2). These two mutations, collectively account for 84.3% of the published cases, with type-1 representing 97% of all 52 bp deletions and type-2 representing 95% of all 5 bp insertions. Standardized HRM analysis readily differentiated mutant samples melting at different temperatures than the homoduplex products (wild type), resulting in altered shapes of the melting curves. We designed sensitive and robust Taqman® Real-Time PCR assays for positive identification of each of the two most abundant types of mutations against the wild type CALR gene. The assays consistently detected 1% of the mutated DNA in 99% background of wild type DNA for both type-1 and type-2 mutations.

Conclusions

We propose a new clinical laboratory diagnostic algorithm for CALR mutations detection based on the standardization of the PCR-HRM analysis and the development of Taqman® Real-Time PCR assays. According to this algorithm, samples can go through front-line screening with fragment analysis or the PCR-HMR method that we describe in this report. Mutations detection, leads to the newly developed Taqman® Real-Time PCR assays for discrimination and quantification of the type-1 and type-2 mutations. In case mutants are not type-1 or type-2, then sequencing should be used for characterization of the genetic alterations. The JAK2 negative MPN samples found to be wild type in the CALR exon 9 should be tested for MPL mutations.

References

[33] Gain of the Paternally Derived DLK1-MEG3 Imprinted Locus in Myeloid Neoplasms with Acquired Uniparental Disomy of Chromosome 14

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⁷UOC di Oncologia e Bone marrow (BM) examination and is based on a major and four minor criteria. The somatic “autoactivating” point mutation D816V in the KIT receptor gene is one of the minor criteria, founded in the great majority of patients (90%) and it plays a central role in the pathogenesis of the disease. Indolent Systemic Mastocytosis (ISM) is the most common variant of SM, characterized by...

[34] Ultra-Deep Sequencing (UDS) allows more sensitive detection of the D816V and other kit gene mutations in systemic mastocytosis

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Objectives and background
Inherited regions of uniparental disomy (UPD) are associated with developmental abnormalities that arise as a consequence of inappropriate expression of imprinted genes or gene clusters (genes that are differentially expressed depending on whether they are maternally or paternally inherited). Acquired UPD (aUPD) is a common finding in haematological malignancies including myelodysplastic syndrome (MDS) and myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and has been associated with acquired mutations of oncogenes or tumour suppressor genes within the regions of UPD; e.g. UPD of chromosomes 4, 7 and 11 has been associated with mutations in TET2, EZH2 and CBL, respectively. Inherited maternal UPD for 14q is associated with Temple Syndrome and involves the DLK1-MEG3 imprinted domain at 14q32 (1). The methylated paternally derived chromosome expresses the protein-...
a very low MC burden and associated with very different clinical pictures. A highly sensitive diagnostic methods for D816V detection are required to assure an appropriate diagnosis and to reduce false-negative results. The recent development of "ultra-deep amplicon sequencing" (UDS) technologies has opened the way to a more accurate characterization of molecular aberrations with higher sensitivity of screening for known and unknown mutations. Our aims were: i) to set-up and optimize a UDS-based mutation screening strategy of the KIT gene on the Roche GS Junior Instrument; ii) to test the sensitivity of our UDS assay to detect the D816V mutation; iii) to investigate the presence of additional KIT mutations in SM.

Methods
We decided to take advantage of a next generation sequencing approach to perform an UDS KIT gene mutation analysis on 20 bone marrow (BM) samples from patients with ISM that were negative for the D816V mutation by Sanger Sequencing which has a sensitivity of 20%. Fusion primers were designed to generate ten partially overlapping amplicon covering the whole KIT transcript (exons 1-21) by RT-PCR. To determine the lower detection limit of our UDS-assay, serial dilutions of the HMC-1 cell line (harboring the D816V mutation) into an unmutated K562 cell line in ratios such as to simulate the following mutation loads were sequenced: 50%, 37.5%, 25%, 12.5%, 5%, 2.5%, 1.25%, 0.5%, 0.25%.

Results and significance
UDS of cell line dilutions showed a high accuracy of D816V mutation detection and linearity of mutation calling over the entire range down to 0.25%. The UDS technology allowed to detected the D816V mutation, below the lower detection limit of Sanger Sequencing, with an abundance from 0.5% to 11%, in 12/20 ISM patients. Two additional sequence variations were detected in a large proportion of patients. These two variations included a 3bp in-frame deletion in exon 15 (GenBank X06182:1: c.2164_2166delAGC; p.S715del) found in 11/20 patients and a 12bp in frame-deletion in exon 9 in all patients, with an abundance ranging from 83% to 97% (GenBank X06182:1: c.1550_1561delGAAACAACAAAG; p.G510_K513del). Previously published studies indicate that the KIT Gly-Asn-Asn-Lys 510-513+/- alternatively spliced located immediately downstream to the extracellular KIT domain and KIT Ser+75+, an interkinase KIT domain, are expressed in normal human hematopoietic cell, leukemic cell lines, acute myeloid leukemia blast and GISTs and represent rather a splice variant of KIT transcript. Interestingly our results showed the presence of the transmembrane domain M541L (GenBank X06182:1: c.1642A>C; p.Met541Leu) KIT-activating mutation in exon 10, with an abundance of 50%, in addition to D816V, in 2/20 ISM. This mutation is known to retain sensitivity to imatinib mesylate7.

Conclusions
Our preliminary results suggest that our UDS-based KIT gene mutation screening assay might be a reliable and sensitive alternative to conventional sequencing methods for the detection of the D816V. We are now planning to investigate whether the greater sensitivity of UDS allows to detect the D816V mutation in peripheral blood mononuclear cells from patients with a suspected clonal mast cell disorder. These results could represent a starting point to plan other extensive studies to better understand the exact role of KIT receptor alterations in SM.

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References

[35] Type and Frequency of Chromosomal Aberrations and Importance of Molecular JAK2V617F Marker in Primary Myelofibrosis

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Objectives and Background
Primary Myelofibrosis (PMF) is a chronic, malignant hematological disease, characterized by leukoerythroblastic blood picture, anisopikilocytosis teardrop-shaped erythrocyte, different degree of bone marrow fibrosis and hepatosplenomegaly due to extramedullary hematopoiesis. Among genetic specificities of the disease, those that stand out are chromosomal aberrations in pathological, myeloid blood cells and point mutation V617F in the JAK2 gene.

The main goal of study was to examine karyotype and cytogenetic parameters and presence of JAK2V617F mutation in the genome of patients with de novo PMF. Additionally, other diagnostic parameters, their mutual correlations and their effect on cumulative survival rate of patients were examined.

Methods
Karyotype analysis was performed by conventional cytogenetic method. Allele-specific PCR was used to detect the JAK2V617F mutation. The study used descriptive and analytical statistical methods. Results and significance: By retrospective analysis of cytogenetic results that included 61 patients, abnormal karyotype was registered in 41% of them. Specific PMF aberrations that were found are: 13q-, 20q-, +8, but also aberrations that are rarely present in this disease. Prospective study included 144 patients. The frequency of chromosomal aberrations was tested, so as the frequency of JAK2V617F mutation, their mutual correlation and correlation with clinical and hematolaboratory parameters.

Results
Chromosomal aberrations were present in 29% of patients. Of specific aberrations for PMF, the most common was trisomy of chromosome 9, then 13q-and 20q-. JAK2V617F mutation was registered in 55% of patients. Examining
the correlation between mutation and type of karyotype and mutation and chromosomal aberrations with various risk level, statistically significant difference was not registered (p=0.153). Examining the importance of clinical and hemato-laboratory parameters, difference was registered in survival of patients with different prognostic groups applying Lille, Cervantes, IPSS, DIPSS cytogenetic prognostic systems (PSs) (for all p<0.001), Mayo PS for all patients (p=0.001) and Mayo PS for younger patients (p=0.013). Testing the influence of the JAK2V617F mutation, it was noticed that there is no statistically significant difference (p=0.807) in the survival of patients with and without mutations. Examining the importance of pathological karyotype and some chromosomal aberrations upon survival of patients, statistical significance (p=0.004) was registered using DIPSS cytogenetic prognostic system (CPS). Applying Lille, Mayo and IPSS CPS, statistic significance was not registered (p=0.155 and p=0.214, p=0.152).

Conclusions

Our results indicate that the overall frequency of chromosomal abnormalities in patients with PMF is 32%. The most common chromosomal aberrations are: +8, +9, 13q- and 20q-. The incidence of JAK2V617F somatic mutation is 55% in our PMF patients cohort. Correlation between chromosomal aberrations and JAK2V617F mutation was not found. All applied PSs are relevant to examine the impact of clinical and hemato-laboratory parameters on survival of patients with PMF. Analysis of prognostic significance of cytogenetic data, performed within several PSs, showed that cytogenetics has a significant impact on survival within the DIPSS score system. Our study suggests the clinical usefulness of simple DIPSS scoring system in presenting and analyzing cytogenetic data in PMF.

[36] Analysis of Factors Associated with the Development of Non-Melanoma Skin Cancer in Patients with Essential Thrombocytopenia and Polycythemia Vera

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Objectives and background

Recent studies have reported an increased risk of new non-myeloid cancer in patients with essential thrombocytopenia (ET) and polycythemia vera (PV) as compared to the general population[1-3]. Moreover, an increased incidence of non-myeloid malignancies has been observed in patients with ET and PV who are homozygous for the minor allele of a single nucleotide polymorphism in the XPD gene (SNP K751Q) [4]. The aim of the present study is to retrospectively analyze the clinical and genetic factors associated with the development of non-melanoma skin cancer (NMSC) in patients with ET and PV.

Methods

A total of 452 patients diagnosed with ET/PV between 1973-2012 in 6 Spanish hospitals were included. Median follow-up of the series was 8.3 years (range: 0.1-37 years). Overall, 51 patients had developed NMSC (basal cell: 28, squamous cell: 22, both types synchronously: 1) after a median time of 6.2 years from ET/PV diagnosis (range: 0.1-23). We analyzed the clinical characteristics (age, gender, geographic area of residence, JAK2 mutational status, treatment) and the genetic markers (SNPs in the NER [XPC8, XPCE15, XPD, ERCC3, ERCC4, ERCC6, ERCC8, XPA, RPA1, RPA2, RPA3], BER [XRCC1], and JAK-STAT [JAK2] pathways) associated with the development of NMSC.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 51)</th>
<th>Controls (n = 401)</th>
<th>p</th>
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<tbody>
<tr>
<td>ET/PV</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>29 (57%)</td>
<td>22 (42%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (&lt;40%)</td>
<td>70 (29-96)</td>
<td>40 (19-93)</td>
<td>0.801</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>27 (53%)</td>
<td>24 (47%)</td>
<td>0.133</td>
</tr>
<tr>
<td>Residence below latitude 40° North</td>
<td>27 (53%)</td>
<td>24 (47%)</td>
<td>0.133</td>
</tr>
<tr>
<td>Hgb, g/L*</td>
<td>137 (96-214)</td>
<td>145 (82-238)</td>
<td>0.008</td>
</tr>
<tr>
<td>JAK2 mutation</td>
<td>36/65 (60%)</td>
<td>242/333 (74%)</td>
<td>0.64</td>
</tr>
<tr>
<td>JAK2 genotype (n=133)</td>
<td>0.023</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCG/CG</td>
<td>34/44 (71%)</td>
<td>39/564 (85%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>16/4/29%</td>
<td>50/764 (13%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Clinical and genetic characteristics that were shown to be different between patients who developed non-melanoma skin cancer (cases) and those who didn’t (controls)
Results
Table 1 summarizes the clinical and genetic characteristics that were shown to be statistically different between patients who developed NMSC and those who didn’t. Of note, no significant association was found between the SNPs in DNA repair genes and the risk of NMSC. At multivariate analysis, factors associated with an increased cumulative incidence of NMSC were age (5% increased risk per additional year, P<0.001), male sex (91% increased risk, P= 0.022), and prior exposure to hydroxyurea (HU) (22% increased risk, P= 0.065). Time of exposure to HU was marginally associated with a higher cumulative incidence of TCNM.

Conclusions
The risk of developing NMSC in patients with ET and PV is not associated with the SNPs in DNA repair genes that were selected in our study. By contrast, such risk is determined by the age and gender of the patient, as well as by the prior exposure to HU.

References

Objectives and background
Aspirin responsive erythromelalgic and migraine-like microvascular ischemic attacks (MIAs) are the main presenting symptom of thrombocythemia in patients with essential thrombocythemia (ET) and polycythemia vera (PV) at platelet counts above 400x10^9/L. Skin punch biopsies taken from the affected areas of erythromelalgia and acrocyanotic complications show typical arteriolar inflammation, fibromuscular intimal proliferation followed by occlusive platelet thrombi in the endarteriolar circulation. If left untreated thrombocythemia patients are at high risk of both microvascular ischemic peripheral, cerebral and ocular ischemic manifestations and major arterial thrombosis. The high thrombotic risk in thrombocythemia decreases to less than 2% per 100 patient-years by primary prevention with low dose aspirin. Such low thrombotic risk by primary prevention with low dose aspirin of microvascular thrombosis applies for low, intermediate and high MPN disease burden, is not age dependent. Both microvascular and major thrombosis do recur when not on low dose aspirin during follow-up. Von Willebrand factor (VWF) mediated platelet thrombi formation, as well as increased proteolysis of the VWF multimers in one and the same patient do occur simultaneously or in sequence leading to the paradoxical occurrence of thrombosis and bleeding at platelet counts above 1000x10^9/L due to an acquired von Willebrand disease type 2A. The stratification as low, intermediate and high thrombotic risk in the retrospective.

Methods
Bergamo studies has been performed in ET patients not on aspirin at time of diagnosis and treated with aspirin for the secondary prevention of major thrombosis in ET and PV patients during follow-up.

Results
The Bergamo definition of low, intermediate and high thrombotic risk for the secondary thrombosis prevention in the 2012 International Prognostic Score of Thrombosis in ET (IPSET) lead to significant overtreatment with hydroxyurea as a significant number of so-called high thrombotic risk ET and PV do in fact have low myeloneoproliferative (MNP) disease burden. According to IPSET guidelines in ET, PV and MNP review papers there is a global overtreatment of ET and PV patients with hydroxyurea. Activated leukocytes are innocent bystanders in promoting the risk of platelet-mediated microvascular ischemic and thrombotic complications in JAK2V617F mutated ET and PV. MNP disease burden in patients with JAK2V617F positive ET and PV is related to JAK2 allele burden and associated with leukocytosis, thrombocytosis, constitutional symptoms and splenomegaly.

Conclusions
Increased platelet count (thrombocythemia) and leukocytes (leukocytosis) above 15 x10^9/L is best treated by low dose Pegasis 45 ug once per week or once per two week in correcting leukocytes to normal and platelet to near normal or normal to postpone the use of hydroxyurea as long as possible. This is associated with reduction of thrombosis risk to near zero, with reduction of enlarged spleen sizes, and above all prevents splenomegaly in early and intermediate stage PV patients during very long-term follow-up.

References


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Abstract

Bone marrow histology differentiates essential thrombocythemia (ET), polycythemia vera (PV) and primary megakaryocytic granulocytic myeloproliferation (PMGM) from all variants of primary or secondary erythrocytosis and reactive thrombocytosis with a sensitivity and specificity of 100%. Bone marrow morphology, serum EPO level and JAK2 allele mutation load cannot discriminate between ET and prodromal PV versus classical PV. A typical JAK2 mutated MPN bone marrow histology with erythrocytes above 5.8x10^12/L in males and above 5.5x10^12/L in females (normal cut-off value is 5.5x10^12/L) separates PV from ET and prodromal PV obviating the need of red cell mass (RCM) measurement. Erythrocytes remain increased at values above 6x10^12/L in PV in complete hematological remission by phlebotomy but hemoglobin and hematocrit values normalize as the consequence of micro-erythrocytosis due to iron deficiency. The combination of increased RCM, increased plasma volume, and normal or low erythrocyte counts is characteristic for Inapparent PV (IPV) due to significant splenomegaly as the cause of increased RCM in the absence of hypervolmicmic symptoms. Six sequential phenotypes of JAK2 mutated MPNs include: JAK2V617F positive heterozygous ET, prodromal PV, hetero/homozygous mutated PV, masked PV, PV-MF and IVP. JAK2 exon 12 JAK2 mutated MPN usually present as idiopathic erythrocytosis or early stage PV. JAK2 wild MPL 515 mutated ET is a distinct clonal MPN entity in which the megakaryocytes are larger and giant with hyperlobulated staghorn-like nuclei without features of PV in blood and bone marrow. Bone marrow histology in CALR mutated ET and MF revealed a typical PMGM picture showing dysmorphic immature megakaryocytes with cloud-like nuclei, which are not seen in MPL 515 mutated ET and also not in JAK2V617F mutated ET, prodromal PV and PV.

[38] Increased erythrocytes on top of bone marrow histology, low serum EPO level and JAK2 mutation screening discriminates JAK2V617F mutated essential thrombocytemia (ET) from polycythemia vera and the importance of bone marrow histology in ET of various molecular etiology

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10On behalf of the European Collaborations and Research on Myeloproliferative Neoplasms: ECAR.MPN.

[39] The PVSG, Rotterdam, Hannover, Cologne and European Clinical Molecular and Pathological (ECMP) criteria for BCR/ABL-negative myeloproliferative disorders (MPD 1975-2008) and the 2014 WHO-CMP classification of five distinct clonal myeloproliferative neoplasms caused by a JAK2, MPL or CALR driver mutation

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9On behalf of the European Collaborations and Research on Myeloproliferative Neoplasms: ECAR.MPN.

Objectives and background
The BCR/ABL fusion gene or the Ph1-chromosome in the t(9;22)(q34;q11) exerts a high tyrosine kinase activity, which is the cause of chronic myeloid leukemia (CML). The 1990 Hannover Bone Marrow Classification separated CML from the myeloproliferative disorders (MPDs) essential thrombocytemia (ET), polycythemia vera (PV) and chronic megakaryocytic granulocytic myeloproliferation (CMGM). The 2006-2008 European Clinical Molecular and Pathological (ECMP) criteria discovered 3 variants of thrombocytectemia: ET with features of PV (prodromal PV), “true” ET and ET associated with CMGM. The 2008 WHO-ECMP and 2014 WHO-CMP classifications defined JAK2V617F three phenotypes of JAK2V617F mutated ET: normocellular ET (WHO-ET), hypercellular ET due to increased erythropoiesis (prodromal PV) and ET with hypercellular megakaryocytic-granulocytic myeloproliferation (ET-MGM). The JAK2V617F mutation load in heterozygous WHO-ET is low and associated with normal life expectancy. The hetero/homozygous JAK2V617F mutation load in PV and myelofibrosis (MF) is related to MPN disease burden in terms of constitutional
symptoms, bone marrow hypercellularity and myelofibrosis. JAK2 exon 12 mutated MPN presents as idiopathic erythrocythemia (IE) and early stage PV. According to 2014 WHO-CMP criteria JAK2 wild type MPLL315 mutated ET is the second distinct thrombocythemia characterized by clustered giant megakaryocytes with hyperlobulated stag-horn-like nuclei, in a normocellular bone marrow consistent with the diagnosis of ‘true’ ET. JAK2/MPL wild type, calreticulin (CALR) mutated hypercellular ET appears to be the third distinct thrombocythemia characterized by clustered large immature dysmorphic megakaryocytes and bulky (bulbous) hyperchromatic nuclei consistent with CMGM or primary megakaryocytic granulocytic myeloproliferation (PMGM).

Conclusion
The 2014 WHO-CMP criteria define three phenotypes of JAK2W617F mutated MPNs ET, prodromal PV, prodromal PV, EMGM and classical PV versus the JAK2 exon 12 mutated idiopathic erythrocythemia (IE) and PV. MPLL15 mutated JAK2 wild type ET and MF is a distinct thrombocythemia without features of PV in blood and bone marrow. CALR mutated JAK2/MPL wild type ET and MF is the third thrombocythemia entity with characteristic features of PMGM in the bone marrow, which are not seen in JAK2 and MPL mutated MPNs. MPN disease burden is best reflected by the degree of anemia and splenomegaly on top of mutation allele burden, bone marrow cellularity and increase of reticulin fibrosis.

[40] Autosomal dominant Aspirin Responsive Sticky Platelet Syndrome in Hereditary Essential Thrombocythemia due to a gain of function mutation in the thrombopoietin (TPO) gene

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Objectives and background
Autosomal dominant hereditary Essential Thrombocythemia (ET) due to a gain of function mutation in the TPO gene in a Dutch family is associated with marked increased TPO levels (530 ± 27 vs controls <62 pg/ml) and Sticky Platelet Syndrome (SPS Kubish et al Sem Thromb Hemostas 2014) with typical manifestations of microvascular circulation disturbances including erythromelalgia and atypical transient ischemic attacks. Increase of large platelets and large mature megakaryocytes with hyperploid nuclei and normal cellularity in bone marrow biopsies of Dutch HET family members were diagnostic for autosomal dominant hereditary ET (HET), which was associated with platelet-mediated thrombocytosis (SPS) comparable as has been observed in acquired JAK2W617F, MPLL515 and CALR mutated ET (Michiels 1985-2015). Similar clinical SPS manifestations and ET specific bone marrow findings are described in a Polish HET family caused by an identical gain of function mutation in the TPO gene. A C→G transversion in the splice donor of intron 3 of the TPO gene co-segregated with increased TPO levels in the affected members of the Dutch and Polish HET families. The patients in the Dutch and Polish HET families showed no endogenous erythroid colony (ECC) formation in all affected members with hereditary ET. Four affected members in the first generation of the Dutch HET family, two sisters and one brother of the propositus, showed no further increase of platelet counts, no features of PV, no splenomegaly during life-long follow-up. Three of these four family members in the first generation of the Dutch HET family developed pancytopenia due to advanced myelofibrosis in 2 at the age of 71 and 73 years respectively, and evolution in acute myeloid leukemia in the third case at age 60. These 3 Dutch HET patients were not treated with cytoreductive agents and used life long low dose aspirin to prevent platelet thrombophilia associated with HET.

Conclusion
Increased plasma TPO levels produced by liver cells caused by a gain of function mutation in the TPO gene on chromosome 3q27 selectively induced proliferation and differentiation of polyclonal hematopoietic stem cells to the formation of large megakaryocytes and large platelets leading to the platelet-mediated arterial thrombophilia (SPS) in hereditary essential thrombocythemia (HET). While on low dose aspirin since 1986, all affected HET patients in the Dutch family were free of microvascular ischemic circulation disturbances and major thrombosis at a stable platelet count between 860 and 1280x109/L during life long follow up without the need of myelosuppressive treatment. Spontaneous evolution of normocellular HET into into pancytopenia due to myelofibrosis in two patients and acute myeloid leukemia in a third case from 4 cases at ages around 70 years in the first generation of the Dutch HET family is part of the natural history of polyclonal HET caused by a gain of function mutation in the TPO gene.
[41] Numerical and Structural Chromosomal Abnormalities of Peripheral Blood Cells of Myeloid Origin in Patients with Idiopathic Myelofibrosis

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2Institute of Hereditary Pathology of National Academy of Medical Sciences, Ukraine

Objectives and background
Mutations of JAK2, CALR and MPL genes are revealed in more than 90% of primary myelofibrosis (PMF) patients [1]. Nevertheless, the mutational status doesn’t show the whole picture of genetic changes in PMF and may manifest different clinical picture in patients with the same mutation. Unfavourable cytogenetic changes in bone marrow (BM) are complex karyotype or sole or two abnormalities that include +8, -7/7q-, i(17q), inv(3), 5q-, 12p-, or 11q23 rearrangement [2]. However, the pathological process in PMF is not limited to BM. The aim of the study was to investigate the karyotype of the peripheral blood cells (PBC) derived from myeloid strain of hematopoiesis in patients with myelofibrosis.

Methods
The study group consisted of 28 patients PMF. Six patients were treatment naive, 18 received hydroxyurea previously, and the other 4 patients were pre-treated with interferon-alpha-2b (IFN). The hydroxyurea and the IFN were stopped before the sample collection. The PBC of all 28 patients were cultivated in vitro for 24h with granulocyte-colony stimulating factor (G-CSF) filgrastim and the nutrient medium. The samples of the first 5 patients were cultivated both with and without G-CSF. G-method of differential staining was used following by the light microscopy.

Results
The mitotic activity in the presence of G-CSF was high enough to produce metaphases for cytogenetic analysis in 20 (71%) of PMF patients, even if the bone marrow puncture was dry. Eight patients had no mitotic activity in the samples, including all 4 IFN-pre-treated patients. All non-stimulated with G-CSF samples didn’t produce metaphases. Chromosomal abnormalities were revealed in the blood of 10 patients. The other 10 patients had normal karyotype. Polyclonality was observed in all patients with cytogenetical abnormalities. In 3 patients tetraploid metaphases were revealed, and in all the cases this feature was clonal. The spectrum of karyotypic abnormalities included also trisomy or monosomy of the chromosome 6, deletions and translocations of chromosome 1, deletions of 5q and 20q. The monosomies of the chromosomes 5, 7, 9, 11, 12, 19 and 21 were recurrent.

Conclusions
Many chromosomal abnormalities from filgrastim-stimulated PBC correspond to those revealed in the BM cultures in other studies. A wide spectrum of cytogenetic abnormalities within a patient may reflect the potential of the circulating myeloid cells to produce genetically variable cell progeny and treatment resistance. The polyploidization could be the intrinsic rescue mechanism for the survival of the pathological clones. The lack of the mitotic activity in the PBC of the IFN-pretreated patients needs to be tested in a greater amount of patients, to adjust whether the IFN could effectively inhibit the evolution of the pathological clones in PMF. Numerical changes or the aberrations of the chromosomes 1, 9 and 11 could have connections with expression profiles of MPL, JAK2 and CALR genes.

References

[42] JAK2V617F Complete Molecular Remission in Long-Term Follow-up of Patients With Polycythemia Vera and Essential Thrombocythemia Enrolled in a Phase II Study with Ruxolitinib. Single Center Experience

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Objectives and background
Polycythemia vera (PV) and essential thrombocythemia (ET) are myeloproliferative neoplasms associated with JAK2V617F mutation. Ruxolitinib is a JAK1/JAK2 inhibitor used in PV and ET patients in the phase II INC18424-256 trial. Results of the PV cohort have been reported recently1: with a median follow up of 35 months, the JAK2V617F allele burden (ab) decreased, and the percentage of patients who achieved a reduction ≥50 % at any time during the 1st, 2nd, and 3rd year was 5.9%, 14.7%, and 23.5%, but no patients achieved a complete remission. Our objective was to evaluate the impact of ruxolitinib on the JAK2V617F clone in a subset of long-term treated (5 year) patients.

Methods
In our centre, we enrolled 11 with PV and 13 with ET. The JAK2V617F ab was measured by 2 RTO-PCR methods: according to Lippert (sensitivity 0.8%) and Larsen (sensitivity 0.08%). We also analysed by NGS (Ion Torrent platform) a set of MPN-associated mutations including TET2, ASXL1, IDH1/2, LNK, CBL, SRSF2, EZH2 and MPL at baseline and after 5 years in all patients who had achieved a >25% JAK2V617F ab reduction (n=13). In 3 informative patients, we also analysed the percentage of JAK2V617F homozygous, heterozygous and wild type clones by allele discrimination based on the 46/1 haplotype.

Results
JAK2V617F ab decreased by a mean of 7%, 11%, 19% and 28% at 1, 2, 3 and 5 years. 3/21 (14%) patients, 1 PV and 2 ET, achieved a ≥50% ab reduction after 2 years, and obtained a complete molecular response (CMR) after 5 years. Their mean JAK2V617F ab was 46.6% before treatment, 28.3%, 16.3%, 8.7% and 0% after 1, 2, 3 and 5 year. The JAK2 CMR was confirmed in at least one subsequent sample and by NGS. At last time point, the PV patient
was in complete haematological remission according to ELN criteria, and the two ET patients in partial remission. The two ET patients achieving CMR did not show any additional mutations, while the PV patient presented a TET2 Y867H with an ab of 48.9% and 52%, respectively at baseline and 5 year. No recurrent mutations in genes other than JAK2 were found in all other examined cases. JAK2 V617F/V617F clones were reduced by a mean of 95.5%, JAK2 V617F/WT displayed a variable trend with a mean reduction of 45.54% and JAK2 WT/WT increased significantly (mean 61.43%) at 5 year. Conclusions: we provide evidence that some PV and ET patients can obtain CMR after long term treatment with ruxolitinib, and that ruxolitinib may preferentially target homozygous clones. However, phenotypic persistence of disease even with CMR for JAK2V617F suggest persistence of ancestral clone(s) as in one TET2 mutated PV patient.

Results
V617F/JAK2 mutation was detected in 44/45(97.8%) pts with PV, 16/30(53.3%) pts with ET and 13/27(3,7%) pt with PMF. 538-539del-insL in 12-th exon of JAK2 was found in 1/45(2,22%) patient with PV. W515K/MPL mutation was identified in 1/30(3,33%) pt with ET and 1/27(3,7%) pt with PMF. 2 mutations of EZH2 have been found in 2 individuals with PMF (2/22). Both mutations are located in the 19 exon. The Ile713Thr mutation was detected in the patient with a del(6)(q15) karyotype which is associated with an intermediate cytogenetics risk. This patient subsequently underwent transformation from PMF to myelodysplastic syndrome in 9 months after the disease onset. Another case of mutation harboring TET2 Y867H with an ab of 48.9% and 52%, respectively at baseline and 5 year. No recurrent mutations in genes other than JAK2 were found in all other examined cases. JAK2 V617F/V617F clones were reduced by a mean of 95.5%, JAK2 V617F/WT displayed a variable trend with a mean reduction of 45.54% and JAK2 WT/WT increased significantly (mean 61.43%) at 5 year. Conclusions: we provide evidence that some PV and ET patients can obtain CMR after long term treatment with ruxolitinib, and that ruxolitinib may preferentially target homozygous clones. However, phenotypic persistence of disease even with CMR for JAK2V617F suggest persistence of ancestral clone(s) as in one TET2 mutated PV patient.

Conclusion
Mutations in EZH2 and CBL genes could be assessed as additional prognostic markers of unfavourable prognosis in patients with BCR-ABL negative MPNs with different chromosomal aberrations. The integration of cytogenetic and molecular analyses could be a valuable option for stratification of patients and optimising the treatment strategy.

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[43] Genetic Methods in Diagnostics and Prognosis of Ph-negative Myeloproliferative Neoplasms

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Objectives and background
Genetic mutations result in abnormalities of myelopoietic proteins and lie in the basis of Ph-negative myeloproliferative neoplasms (MPNs) development and its subsequent progression. Several somatic mutations in JAK2, MPL, TET2, EZH2, ASXL1, CBL, IDH1, IDH2, IKZF1 genes were detected in chronic and blastic phase MPNs. Recent studies have revealed a number of epigenetic alterations that contribute to Ph-negative MPNs pathogenesis and determine the clinical outcome. Mutations involving the EZH2 gene are thought to result in loss of methyltransferase activity suggesting a potential role of tumor suppressor gene silencing as a mechanism in the disease progression. Decrease in ubiquitin ligase activity caused by mutations in CBL gene leads to myeloid proliferation. EZH2, CBL mutations are thought to be of prognostic value in MPN’s at onset. Another case of mutation harboring TET2 Y867H with an ab of 48.9% and 52%, respectively at baseline and 5 year. No recurrent mutations in genes other than JAK2 were found in all other examined cases. JAK2 V617F/V617F clones were reduced by a mean of 95.5%, JAK2 V617F/WT displayed a variable trend with a mean reduction of 45.54% and JAK2 WT/WT increased significantly (mean 61.43%) at 5 year. Conclusions: we provide evidence that some PV and ET patients can obtain CMR after long term treatment with ruxolitinib, and that ruxolitinib may preferentially target homozygous clones. However, phenotypic persistence of disease even with CMR for JAK2V617F suggest persistence of ancestral clone(s) as in one TET2 mutated PV patient.

Methods
We have examined 102 patients with Ph-negative MPNs (45 pts with PV, 30 with ET and 27 with PMF). For all patients the detection of V617F mutation of JAK2 gene was done. V617F-negative pts with PV and pts with ET/PMF underwent the analysis of mutations in 12-th exon of JAK2 and 515 codone of MPL respectively. For 80 pts (30 with PV, 26 with ET and 22 with PMF) cytogenetic analysis and EZH2 mutation status testing were performed. Identification of CBL mutations was performed in 24 patients with available RNA samples. Mutations in 8,10,17,18,19 exons of EZH2 and RING-domen of CBL were defined by sequence analysis.

Results
V617F/JAK2 mutation was detected in 44/45(97.8%) pts with PV, 16/30(53.3%) pts with ET and 13/27(3,7%) pt with PMF. 538-539del-insL in 12-th exon of JAK2 was found in 1/45(2,22%) patient with PV. W515K/MPL mutation was identified in 1/30(3,33%) pt with ET and 1/27(3,7%) pt with PMF. 2 mutations of EZH2 have been found in 2 individuals with PMF (2/22). Both mutations are located in the 19 exon. The Ile713Thr mutation was detected in the patient with a del(6)(q15) karyotype which is associated with an intermediate cytogenetics risk. This patient subsequently underwent transformation from PMF to myelodysplastic syndrome in 9 months after the disease onset. Another case of mutation harboring TET2 Y867H with an ab of 48.9% and 52%, respectively at baseline and 5 year. No recurrent mutations in genes other than JAK2 were found in all other examined cases. JAK2 V617F/V617F clones were reduced by a mean of 95.5%, JAK2 V617F/WT displayed a variable trend with a mean reduction of 45.54% and JAK2 WT/WT increased significantly (mean 61.43%) at 5 year. Conclusions: we provide evidence that some PV and ET patients can obtain CMR after long term treatment with ruxolitinib, and that ruxolitinib may preferentially target homozygous clones. However, phenotypic persistence of disease even with CMR for JAK2V617F suggest persistence of ancestral clone(s) as in one TET2 mutated PV patient.

Conclusion
Mutations in EZH2 and CBL genes could be assessed as additional prognostic markers of unfavourable prognosis in patients with BCR-ABL negative MPNs with different chromosomal aberrations. The integration of cytogenetic and molecular analyses could be a valuable option for stratification of patients and optimising the treatment strategy.

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Objectives and background
Young adults with Essential Thrombocythemia (ET) or early Primary Myelofibrosis (early-PMF) are a category of patients projected to a prolonged utilization of medical resources; however, few data are available on their long-term outcome.1

Methods
A clinic-pathologic database of ET patients followed in four Italian Hematology Centers was created. A total of 215 WHO-diagnosed2 ET or early-PMF patients ≤ 40 years at diagnosis was retrieved from the general database of 2635 patients. Baseline clinical/molecular characteristics and outcome measures (vascular complications, disease transformation/progression, overall and event-free survival) were evaluated.

Results
Overall, 197 WHO-defined ET and 18 early-PMF (age range: 16-40, median 34) were included in the study. Overall, 128 patients (60%) carried the JAK2V617F mutation; 48 patients (23%) were CALR mutated and 11 (5%) were MPLW515L positive; in the remaining 28 patients, no mutation was detected. No difference were observed between ET and early-PMF patients in terms of baseline clinical characteristics, a part from a higher incidence of early-PMF in women. Also, use of antiplatelet and of cytoreductive therapies was comparable in the two groups. Median follow-up was 10.2 years (range: 0.5-37.5). During follow-up, 19 (9.6%) ET and 2 (11%) early-PMF patients experienced a total of 31 thrombotic (arterial: 38%) and 12 hemorrhagic events, with an incidence rate of 0.89% and 0.37% patients/yr, respectively. The cumulative incidence of thrombosis was 0.14% and 0.20% at 15 and at 20 years, respectively. Overall, 10 patients (4.6%) and 1 (0.4%) patients evolved to MF and AL, respectively, 11 developed a second neoplasia. The cumulative incidence of disease progression was 0.06% and 0.15% at 15 and at 20 years, respectively. At last contact, 6 (2.7%) patients had died, at a median age of 61 years (20-71), for an overall survival of 96% at 15 years. Causes of death were related to the hematological malignancy, pointing out the substantial impact that this generally indolent disease may acquire in young adults.

Conclusions
With the limitations due to the retrospective nature of the study, the outcome of young adults with early-PMF and true ET seemed to be comparable. The correlation of abnormal karyotype with MF transformation suggests the need for an accurate cytogenetic analysis at diagnosis. Although the number of events was low throughout the follow-up, causes of death were mostly related to the hematological malignancy, pointing out the substantial impact that this generally indolent disease may acquire in young adults.

References
Objectives and background
Polycythemia Vera (PV) is one of the common chronic myeloproliferative diseases. Results of recent clinical trials gave encouraging results for target therapy introduction. The objective of our study was to analyze incidence, symptoms and therapy results to develop optimal PV management algorithm in our center.

Methods
We have obtained and analyzed information about primary incidence, diagnostic methods results, treatment options, thrombosis rate and survival. We have analyzed risk of thrombosis in patient groups, have divided according to IPSET thrombosis prognostic scale. Response to therapy was evaluated according to ELN criteria.

Results
Representative sample consisted of 252 patients (145 female, 107 male). Annual primary incidence for the last 10 years (from 2004 to 2013) varied from 0.5 to 1.15 with mean of 0.83 new patient per 100 000 inhabitants. The median age of population was 59 years (range 20 – 86). The most prevalent initial disease symptoms were: plethora (85%), headache and dizziness (60%), fatigue (27%), pruritus (21%), arthralgia (7%), erythromelalgia (5%), 3% of patients had no symptoms. Diagnostic CBC data (mean (95%, CI)) were as follows: HB 18.7 (13.1-25.6) g/dl, RBC 7.17 (5.17-10.29) x 1012/l, HCT 59.1 (43.0-79.0), WBC 11.7 (3.6-64.8) x 109/l, PLT 509 (136-1642) x 109/l. Bone marrow fibrosis grade 0 were noted in 91.4%, grade 1 in 2.9%, grade 2 in 5.7% of patients. Cytogenetic abnormalities were not seen in any of 0/18 of patients. JAK2V617F was detected in 97.7% patients, JAK2 mutations in 12 exon was revealed in 2.3% patients. Patients were treated mainly with hydroxycarbamide – 81.8% (mean daily dose was 0.7 g), interferon-alpha – 17.1% (mean weekly dose was 8.5 million IU), mercaptopurine – 10.1% patients. Erythrocytapheresis procedures were performed in 88.9% patients, with rate from 0.10 to 8.52 (mean 2.84) procedure per year. Responses to therapy were: complete clinicohematological response in 7.5%, partial response in 72.6%, no responses were seen in 19.8% patients. Thrombotic complications occurred in 28 (11.1%) of patients (16 arterial and 13 venous thrombotic episodes). Myocardial infarction was found in 9 (3.6%), cerebrovascular accident in 13 (5.2%) patients. Thrombosis rates in WHO IPSET-thrombosis system risks’ groups were: low-risk group 2.6% (2/78), intermediate-risk group 7.8% (6/77) and 20.6% (20/97) in high-risk group with significant (p<0.0004) differences between risks’ groups. There were 56 lethal outcomes in the analysed group. Overall survival was 77.7% (fig.1); actuarial median survival was 20.2 years.

Conclusions
PV is one of the most frequent myeloproliferative neoplasms. Contemporary diagnostic methods and disease prognostic systems allow us to improve quality of therapy. Taking into account low rate of complete responses and high rate of thrombosis in high-risk patients, new therapeutic options are necessary.
AML ABSTRACTS

Background
Abnormalities in the control of apoptosis play an important role in tumorigenesis. Survivin is one of eight members of the inhibitor of apoptosis protein family (IAP) that regulates, integrates cell division and suppresses apoptosis. Survivin shuttles between the nucleus and the cytoplasm; it effectively inhibits apoptosis, by binding to second mitochondrial activator of caspase. Expressed during embryonic development and by many cancer cell types, but not in the differentiated normal tissue, survivin is implicated in control of cell survival and regulation of mitosis in cancer.

Objectives
To assess expression of survivin in Egyptian AML patients and its correlation to outcome, progression, and survival.

Methods
120 patients with AML (52 females and 68 males) were recruited and followed up for 2 years (mean age: 42.1 ± 13.1 years). 16 patients had AML-M0, 32 AML-M1, 32 AML-M2, 12 AML-M3, 16 AML-M4 and 12 AML-M5 (FAB classification). A control group included 60 volunteers. Detection of intracellular Survivin antigen in myeloid blast cells was done by flow cytometry on bone marrow samples at D0 and D28.

Results
Survivin expression was higher in AML patients at D0 and D28 compared to healthy controls (P=0.001), highest survivin level seen in AML M5 (FAB subtypes) followed by M4, then by M1, M0, M3 and M2 subtype. A statistically significant positive correlation was found between age of patient and Survivin expression, and between survivin level at D0 and at D28 among responders (P=0.001), also between D0-survivin level of AML patients with favorable response compared to patients with unfavorable response (P=0.005), survivin expression positively correlated with CD15, CD14 and CD11c expression. There was statistically significant negative correlation between survivin level and with Complete remission (CR), overall survival (OS), EFS, and PFS. Survivin expression was higher in elderly AML patients compared to younger patients. A statistically significant negative correlation was detected between survivin level at D0 and CR, OS, EFS, and PFS. Survivin is an attractive therapeutic target to inhibit cancer growth by inhibiting extrinsic and intrinsic apoptotic pathways and confers resistance to apoptosis by directly suppressing caspase activity.

Conclusions
Survivin is important factors involved in control of apoptosis in malignant cells. Survivin expression was higher in elderly AML patients compared to younger patients. A statistically significant negative correlation was detected between survivin level at D0 and CR, OS, EFS, and PFS. Survivin is an attractive therapeutic target to inhibit cancer growth by inhibiting extrinsic and intrinsic apoptotic pathways and confers resistance to apoptosis by directly suppressing caspase activity.

References
[47] Acute promyelocytic leukemia (AML 3): About 36 cases

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Introduction
Acute promyelocytic leukemia (APL) is currently the only acute leukemia whose prognosis was highly Blvd everted since the advent of targeted therapy based on acid all-trans - retinoic acid (ATRA) has made this highly curable disease.

Objectives
To describe the epidemiological, clinical, biological and therapeutic results of the LAP.

Methods
It is a retrospective study of 28 cases of LAP collated in the haematology department at the University Hospital of Sousse Farhat HACHED over a period of 08 years (2004-2013).

Results
The median age of patients was 35.5 years (7-58 years), 61.1% of patients were female with a sex ratio of 1:5.7. The average consultation time was 21 days. The main reason for consultation was a hemorrhagic syndrome of varying intensity. The disease was discovered in a patient during a stroke. The diagnosis is made after cytology, cyto genetics and molecular analysis. According score SANZ, 12 patients had a high risk and 4 had low risk. Biological DIC was present in 19 patients. 2 patients had micro granular form. The karyotype was in a normal case, showed the t (15:17) in 31 patients with various other in case of abnormalities. The Search for the PML / RARA mutation was performed in 28 patients. Our patients received chemotherapy according to the AIDA protocol. 4 patients had ATRA syndrome. CR was obtained in 86.11% of cases, 5 patients died during induction of hemorrhagic syndrome in three cases of myocardial infarction in a diabetic patient with hypertension and severe sepsis in one case. Three relapses summers noted, being caught by the maintenance chemotherapy followed by an allograft in one case treatment. Overall survival at 5 years is 62 %.

Conclusion
Since the introduction of ATRA, there has been remarkable progress in the treatment of APL. Arsenic is currently used to treat relapses successfully to produce hematopoietic stem cells in second complete remission.

[48] Invasive Aspergillosis in Adult Patients with Acute Myeloid and Lymphoblastic Leukemia in Saint Petersburg, Russia

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Introduction
Invasive aspergillosis (IA) is a major complication in patients with acute leukemia.

Objectives and background
Analysis of demographic parameters, risk factors, etiology, treatment and survival rates in adult patients with acute myeloid and lymphoblastic leukemia, and IA.

Methods
Prospective study in 1998-2013 yy. We used criteria EORTS/MSG, 2008 for the diagnosis of proven and probable IA. For identification of prognostic factors multifactorial analysis was used.

Results
We observed 176 patients with proven and probable IA. Group I included 116 patients with acute myeloid leukemia (AML), males - 51%. Group II - 60 patients with acute lymphoblastic leukemia (ALL), males - 68%. In the AML group mean age of patients was 39.5 years (range 18-78) vs ALL group – 29 years (range 18-68), (p = 0.02). According to EORTS/MSG, 2008 criteria, 92% of AML patients had the probable and 8% proven IA, ALL – 90% and 10%, respectively. IA was diagnosed after allogeneic hematopoietic stem cells transplantation in 33% vs 52% patients (p = 0.04). Neutropenia (<0.5×109/L) was observed in AML patients - 90% (median - 17 days), in ALL patients - 88% (median 13 days) before IA development. Bacterial or viral infections were detected in 46% vs 40% patients at the time of IA diagnosis. The main sites of infection in both groups were lungs – 98% vs 96%, other localization was observed predominantly in combination with lung injury: sinuses – 4% vs 10%, central nervous system – 3% vs 10%. Galactomannan test in bronchoalveolar lavage fluid (BAL) was positive in 64% vs 71% cases, direct microscopy of BAL was positive in 27% vs 33% samples. Aspergillus spp. were isolated in culture in 27% vs 25% cases. The main etiological agents were: A.fumigatus - 56% vs 54%, A.flavus – 24% vs 21%, A.niger – 20% vs 21% in AML and ALL groups. A.versicolor, A.nidulans, and A terreus were detected rarely. Mixed Aspergillus infection was found in 13% vs 25% cases, respectively (p = 0.03). All patients received antifungal therapy: voriconazole – 73% vs 86%, caspofungin – 16% vs 18%, amphotericin B deoxycholate - 9% vs 16%, and posaconazole - 7% vs 5%, respectively in AML and ALL groups. Secondary antifungal prophylaxis was conducted in 35% vs 37% patients. Overall survival rate in twelve weeks was 77% vs 73%. Positive prognostic factors of 12th week survival in both groups were bronchoscopy use for the early IA diagnosis (p = 0.01), antifungal treatment with voriconazole (p=0.002), and secondary antifungal prophylaxis (p=0.001). Negative prognostic factors were underlying disease relapse (p = 0.02), bacterial or viral infection at the time of IA development (p = 0.03), and disseminated IA (p = 0.04).

Conclusions
In adult patients with acute myeloid and lymphoblastic leukemia main risk factors of invasive aspergillosis were neutropenia and allogeneic hematopoietic stem cells transplantation. The main etiological agents were A. fumigatus, A. flavus, and A. niger. Lung involvement was in most cases. Twelve week overall survival was 77% and 73%, respectively. Positive prognostic factors of 12th week survival were bronchoscopy use for the early diagnosis, treatment with voriconazole and secondary antifungal prophylaxis.
[49] Invasive Aspergillosis in Children with Acute Lymphoblastic and Acute Myeloid Leukemia in Saint Petersburg, Russia

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Objectives
To analyze demographic parameters, underlying diseases, etiology, treatment and survival rate of invasive aspergillosis (IA) in pediatric patients with acute lymphoblastic leukemia and acute myeloid leukemia in age.

Methods
Prospective study during 1998-2014 y. We analyzed 67 hematology patients under 18 years old with “proven” and “probable” IA from 4 hospitals in Saint Petersburg. Diagnosis of IA was made according to EORTC/MSG criteria (2008). "Platelia Aspergillus", BioRad test was used for galactomannan (GM) detection in serum and bronchoalveolar lavage fluid (BAL).

Results
We examined 43 patients with acute lymphoid leukemia (ALL), the median age was 11 years (range 1-18), males - 54%; and 23 patients with acute myeloid leukemia (AML), median age - 11 years (range 2-18), males - 61%. “Proven” IA was diagnosed in 7% ALL patients vs 8% AML, “probable” - 93% vs 92%, respectively. Patients in both groups received cytostatic therapy, the average number of courses - 6 vs 3, respectively (p=0.025). Neutropenia (<0,5x109) >10 days was main risk factor of IA in 75% ALL patients (median - 27 days) vs 65% AML (median - 21 days). Allogeneic hematopoietic stem cells transplantation was made in 49% ALL patients vs 50% AML. The main sites of infection in both groups were lungs, 88% ALL patients vs 100% AML (p=0.04). Respiratory failure was observed in 25% ALL patients vs 61% AML (p=0.04). Sinus involvement was in 7% ALL patients. Disseminated aspergillosis (≥2 organs) was observed in 9% ALL patients vs 13% AML. Diagnosis was confirmed by histology of lung biopsy and autopsy in 7% ALL patients vs 8% AML. Bronchoscopy was made in 65% ALL patients. In this group diagnosis was confirmed by positive GM test in BAL - 64%, in serum - 54%. Aspergillus spp. were cultured in 25% BAL: A. fumigatus - 13%, A. niger - 6%, and A. terreus - 6%. Bronchoscopy was made in 54% AML patients. Diagnosis was confirmed by positive GM test in BAL - 60%, in serum - 52%. Aspergillus spp. were cultured in 30% BAL: A. fumigatus - 20%, A. nidulans - 5%, and A. orrhaceus - 5%. All patients were treated with antifungal therapy: voriconazole - 68% vs 60%, amphoterin B - 26% vs 32%, itraconazole - 6% vs 8%, combination antifungal therapy - 12% vs 8%. Twelve weeks overall survival in ALL patients was 67% vs 63% AML. Positive prognostic factor in both groups was secondary antifungal prophylaxis (p=0.003), negative prognostic were simultaneous bacterial or viral infections (p = 0.04), and respiratory failure (p = 0.04).

Conclusions
Neutropenia and allogeneic hematopoietic stem cells transplantation were main risk factors of invasive aspergillosis in children with acute lymphoblastic and acute myeloid leukemia. The most common pathogen was A. fumigatus. The main localization were lungs, and respiratory failure was more common in AML patients (65% vs 25%, p=0.04). Twelve weeks overall survival was 65%. Positive prognostic factor was secondary antifungal prophylaxis (p=0.003), negative prognostic were simultaneous bacterial or viral infections (p = 0.04), and respiratory failure (p = 0.04).

[50] The Prognostic Significance of L- and H- Isoferritins in Acute Leukemia

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Objectives and background
Hyperferritinaemia has been previously described both for various oncopathology and for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsible for a number of hemoblastosis. Over the last years it has been found out that ferritin being an acute phase protein is not only responsibl...
have better chances for complete remission and increased overall survival as a result. L-ferritin level, more than 1500 mkg / ml at the onset of acute leukemia, is an adverse prognostic factor, reflecting the low rate of complete remission and has an impact on reducing the overall survival.

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[51] Appearance of acute myelogenous leukemia (AML) in a patient with breast cancer after adjuvant chemotherapy: Case report and review of the literature

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Objective and Background
Breast cancer is the most frequent cancer among women and the leading cause of death among middle-aged women. Adjuvant chemotherapy, commonly including alkylating agents and anthracyclines improves survival rates in operable breast cancer but treatment-induced acute myelogenous leukemia (AML) is now widely regarded as an important concern for survivors. AML is an aggressive hematologic cancer that caused by abnormal proliferation and accumulation of hematopoietic progenitor cells. According to FAB classification, subtypes of AML are M0 to M7. The aim of this study is to evaluate treatment agents for breast cancer and effect of them on risk of AML incidence.

Case report
a 37-years-old woman was referred to clinic of oncology in Kermanshah, Iran with a self-detected mass and pain in her left breast. Pathology report of biopsy confirmed invasive ductal carcinoma, with immunohistochemical (IHC)-based estrogen receptor (ER) and progesterone receptor (PR) positive results. P53 was also negative, and Ki67 was positive in 50% of tumor cells. Furthermore, human epidermal growth factor receptor 2 (Her-2) was 3+ for her. Tumor stage was IIIA. Status of patient in sentinel lymph node biopsy, bone scan and computerized tomography (CT) scan of abdomen and pelvis were normal. She was sent for radical modified left breast mastectomy and axillary dissection, and she was then treated with combination of anthracycline with cyclophosphamide for four courses, afterward four courses of paclitaxel with trastuzumab for one year (17 courses of trastuzumab). Due to node-positive, she was treated in follow up with irradiation on site of surgery and left axillary area. After 18 months of the first treatment for breast cancer, she came back again to our clinic with gingival hyperplasia complaints. Peripheral blood analyses indicated WBC >40000/µL with immature (blasts) cell and in her bone marrow biopsy, AML (French-American-British (FAB) classification M2) was documented. She was treated with diagnosis of secondary leukemia with 7+3 regimen with complete remission and in continues she received two further courses of high dose Ara-c. She had a full match sibling donor for allogeneic transplant, but unfortunately she rejected procedure of bone marrow transplantation. She died with relapse of AML after six months of last consolidation.

Conclusions
There is strongly this possibility that addition of paclitaxel-therapy (to irradiation and cyclophosphamide) reduce interval between two malignancies (breast cancer and AML).
Objectives and background
Normal karyotype (NK) in AML patients accounts for nearly 45% of all cases and is assigned into intermediate risk group. The identification of new molecular markers in this group is the focus of most of researches. The application of the next-generation sequence techniques led to detect molecular markers with valuable prognostic significance. E.g., identification of DNMT3A mutations has gained the tremendous attention in recent times, because of its essential role in cell development, high frequency in AML patients and association of poor clinical outcome. Objects: to analyse character and frequency of DNMT3A mutations in AML patients; to study their associations with clinical and laboratory parameters and other molecular markers; to investigate their prognostic value.

Methods
The screening of DNMT3A mutations was performed by the high-resolution melting curve analysis. Mutations in FLT3, CKIT and NPM1 were analysed by polymerase chain reaction and in NRAS by sequencing. Standard GTG-method was used for patients karyotyping. The investigation group included 98 AML patients. Missense mutations of DNMT3A exon 23 (R882) were identified in 16 (16.3%) de novo AML patients. The most common mutation in DNMT3A was R882H (n=9; 56.3%), followed by R882C (n=6; 37.5%), and R882S (n=1; 6.2%). All but one patients (with mutation R882S) were heterozygous and retained a wild-type allele. Patients with isolated DNMT3A mutations were seen in 3 cases; 3 pts with R882C had also mutations in NRAS; 3 pts had DNMT3Amut/FLT3-ITDmut; 1 pt - DNMT3Amut/FLT3-ITDmut/FLT3-TKDmut; 4 pts - DNMT3Amut/FLT3-ITDmut/ NPM1mut and 3 pts - DNMT3Amut/ NPM1mut.

Results
Patients who harbored a mutation in DNMT3A had higher white blood cells count (p=0.039) at diagnosis and more frequently belonged to FAB group M4 (p=0.033), as compared with DNMT3A wild-type. Patients with isolated DNMT3A mutations were seen in 3 cases; 3 pts with R882C had also mutations in NRAS; 3 pts had DNMT3Amut/FLT3-ITDmut; 1 pt - DNMT3Amut/FLT3-ITDmut/FLT3-TKDmut; 4 pts - DNMT3Amut/FLT3-ITDmut/ NPM1mut and 3 pts - DNMT3Amut/ NPM1mut.

Conclusions
AML with DNMT3A mutations represent the group, homogeneous on a number of clinical and laboratory parameters. DNMT3A mutations are highly recurrent in patients with de novo AML with an intermediate-risk cytogenetic profile. FLT3-ITD and NPM1 mutations appear as a major significant coexisting genetic mutations in DNMT3Amut pts. The presence of DNMT3A mutations can be considered as independent adverse prognostic factor for OS, suggesting that testing of DNMT3A mutations can help further improve risk stratification in NK-AML.

References

[52] Prognostic Significance of DNMT3A Gene Mutations in Patients with Acute Myeloid Leukemia
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AML ABSTRACTS


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Introduction
Acute myeloid leukemia (AML) is the most common acute leukemia in adults, prognosis was improved in recent years thanks to allogeneic bone marrow transplantation and advances in supportive treatment allowing complete remission in 70 to 80% of the cases.

Objectives
We propose to evaluate the therapeutic management of AML in adults from 2006 to 2011.

Methods
It is a retrospective study of 82 cases of AML in adults less than 60 years over a period of 06 years (2006-2011).

Results
The median age of patients was 41 years (18-60 years), 64.6% of patients were male. The clinical examination associated granulocytic sarcoma in 2 cases, an initial neurological impairment was found in 3 cases. AML is secondary in 3 cases. The rate of white blood cell is > 30 000/mm3 in 45% of cases. According to the WHO classification, the monocytic forms presents 28% of cases, a favorable cytogenetic group in almost 16% of cases, intermediate in 62% and negative in 22% of cases. All our patients received a course of induction-type aracytine + idarubicin (68%), aracytine + daunorubicin (32%), the complete remission rate was 51%, the death rate is 23%. The relapse rate is 20%, 3 patients were grafted with a favorable evolution in one case. At the end of treatment, the complete remission rate was only 28% with a total toxic death rate after 3 cycles of consolidation of the order of 32%. The overall 5 year survival is 60%, and relapse-free survival at 5 years was 50%.

Conclusions
Our results are lower than those reported in the literature, early stratification of patients according to cytogenetic data and molecular biology would be necessary to adapt the treatment, a better parallel processing support and allogeneic bone marrow transplantation for greater number of patients subsequently allow it to improve these results.

[54] Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) for adults with Acute Myeloid Leukemia (AML): the Tunisian results

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Objectives and background
In Tunisia, allogeneic HSCT is recommended for intermediate and poor-risk AML if an HLA-identical sibling donor was available. Here, we report our results with the Bu(iv)-Cy regimen.

Methods
Between January 2005 and December 2013, 85 patients were transplanted for AML. The median age was 33 years (range; 17-49 years). According the cytogenetic stratification, 80 valuable patients were stratified either in the poor-risk (n=31; 39%) or in the intermediate risk (n=44; 55%) or in the favorable risk group (n=5; 6%). Induction treatment was the standard Cytarabine+Mitoxantrone “7+3”. The transplant was performed in first complete remission (CR1) (n = 68), in second CR (CR2) (n=7) or in failure (n=10). Conditioning regimen was Bu (iv) - Cy (97%) or ICT- Cy (3%). GVHD prophylaxis associated Cyclosporine A and methotrexate. The graft was bone marrow in 54 patients (64%) providing a median of 2.2 x108 MNC/ kg (range; 0.7 - 4.07) and peripheral blood stem cells in 31 patients (36%) providing a median of 4.99 x 106 CD34+ cells/kg (range; 2.46 - 6.79).

Results
Engraftment was achieved in 84 patients (99%). Only one early death occurred and was related to a toxic acute renal failure (1%). The rate of overall treatment–related mortality was 8% (n = 7). Causes of death were refractory GVHD and non-infectious pulmonary complications. The cumulative incidence of relapse was of 29%. The median time to relapse was 10 months (range; 2 - 25months). The cumulative incidence of acute GVHD, cytomegalovirus infections and chronic GVHD were 31 %, 36% and 50% respectively. After a median survival of 30 months (range; 20 days -100 months), 59 patients were alive. The overall survival (OS) and the disease-free survival were of 68% and 63% at 3 years respectively. The OS rates were of 75 %, 42 % and 12 % for patients transplanted in CR1 , CR2 and failure respectively (P = .0001 ). Pre-transplant disease status was the only risk factor that significantly affects survival.

Conclusion
Allogeneic HSCT with sibling donor in adult AML patients is relatively safe. The pre-transplant disease status is discriminant for survival.
Acute myeloid leukemia (AML) is a cancer of the myeloid line of blood cells, characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells. Therapeutic efficiency in treatment of AML ranges from 20 to 45%. One of the causes is an acquired drug resistance of leukemic cells after drug treatment. Another and more important cause is a de novo or primary resistance of to induction of cellular death. This form of leukemic cells resistance associated with bone marrow microenvironment such as stroma cells and factors (interleukins, growth factors, CSFs).

Materials and methods
Etoposide, sorafenib and recombinant human izTRAIL (rh izTRAIL) were used as cytotoxic inductors. Human AML THP-1 cells and primary bone marrow mononuclear cells (BMMC) of patients with diagnosed AML were used as cell models. Multicellular aggregates were formed by cell culture on the agarose-coated plates. For disruption of cell-to-cell contact, THP-1 and BMMC were cultivated in the medium containing 0.9% methylcellulose. The viability of cells was assessed by Alamar Blue assay.

Results: In multicellular aggregates, 75±5% of THP-1 cells was resistant to rh izTRAIL, 70±5% – to etoposide, and 40±7% – to sorafenib. Disruption of cell-to-cell contact decreased the drug resistance. In multicellular aggregates of primary BMMC, 45±5% of cells was resistant to sorafenib, 57±4% – to etoposide, and all cells were resistant to izTRAIL. Disruption of cell-to-cell contact decreased the resistance to sorafenib and etoposide but not to rh izTRAIL.

Conclusion
In multicellular aggregates of THP-1 cells and primary bone marrow mononuclear cells of patients with diagnosed AML increase resistance to rh izTRAIL, etoposide, and sorafenib. Disruption of cell-to-cell contact decreases drug resistance of AML cells. We suppose that one of the causes of de novo AML drug resistance can be multicellular aggregations of AML cells in bone marrow.
[56] Efficacy of Oral Chelation Therapy of Myelodysplastic Syndromes Complicated by Transfusion Hemosiderosis

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Objectives and background
Evaluating the clinical efficacy and tolerability of chelation therapy of deferasirox in patient with myelodysplastic syndromes (MDS), dependent on transfusions with serum ferritin levels higher than 1,000 µg/l.

Methods
We have observed 22 patients with a median age of 58 years for 6 months. All patients received at least 8-10 units of red blood cells transfusions (RBCs) in the past 12 months. For patients receiving less than 4 units of RBCs per month, the recommended initial daily dose of deferasirox was 20 mg/kg/body weight. An initial daily dose 30 mg/kg/body weight was recommended for patients receiving more than 4 units of transfusions per month and/or with serum ferritin levels higher than 3,000 µg/l. Tablets were dispersed in water and suspension was taken 30 minutes before food. Iron load was monitored monthly by identifying of the levels of serum ferritin and of labile plasma iron (LPI). Furthermore, levels of creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin was determined once a month.

Results
We have been identified the median serum ferritin level was 4,300 µg/l (range 1,600-10,000) before the start of chelation therapy, and it amounted to 2,800 µg/l (range 420-8,000) after 6 months of treatment. The decreasing of LPI was from 56 µmol/l to 48 µmol/l. The most common adverse events (AEs) that were observed during the first 2-3 weeks of treatment were mild-to-moderate gastrointestinal disturbances - diarrhea in 4 patients, 8 patients had nausea, lack of appetite in 10 patients. These AEs did not require drug discontinuation. Patients were prescribed symptomatic therapy: antidiarrheal agents, pancreatic enzymes, metoclopramide. The dose reducing was performed from 20 mg/ kg to 10 mg/kg in 7 patients within two weeks and from 30 mg/kg to 20 mg/kg in 4 patients during the 2-3 weeks. Treatment reintroduction and return to the previous dose of deferasirox had significantly better tolerability. Clinically significant increase in levels of creatinine, ALT, AST, bilirubin was not revealed. Clear signs of a toxic effect have not been identified.

Conclusions
Thus, the timely administration of oral chelation therapy is highly effective in reducing level of serum ferritin with good tolerability.

References

[57] EMSCO – the European MDS Studies Coordination Office: towards a European MDS clinical trial platform

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Methods
Based on the above prerequisites, the French and German MDS study groups have, with the support of the ELN, taken the initiative in 2012 to set up a single point of contact for cross-European coordination in MDS. A 2-step approach was taken to implement such a study platform: (1) planning for phase 1 included to set up an office located in Dresden and to launch a first common French-German trial in lower-risk MDS. (2) Phase 2 foresaw an extension of the network and its projects in order to create a self-financing organization.

Objectives and background
Enhancing the quality of cancer care throughout Europe is essential for improving cancer survival rates. High quality clinical research will make an important contribution to this goal. Myelodysplastic syndromes (MDS) are relatively rare diseases of the older population primarily presenting with anemia and cytopenia and frequently progressing to acute myeloid leukemia (AML). Clinical research in MDS accelerated in recent years with about 350 trials open worldwide in 2014 – 70 of them in Europe [1]. These efforts are based on an even more detailed understanding of the disease mechanisms, primarily based on cytogenetic findings and the relevance of specific mutations. Current and upcoming clinical research reflects this evolution: trial designs must meet the requirements of increased disease complexity by incorporating increasingly complex stratifications, smaller subgroups and by targeting specific patient groups with tailored therapy options. Thus, it is expected that a significant number of new medications will come into testing for MDS over the next 5-7 years. Such efforts, especially when carried out internationally, require stringent management, coordination and bundling across borders.
Results
According to phase 1 planning an office was set up in 2012 and a name given to the platform: “EMSCO - the European MDS Studies Coordination Office”. Furthermore, EMSCO’s corporate design was established and a web presence launched (www.emsco.eu). Following the logistical set-up, the planning phase for the first EMSCO-run clinical trials (DACOTA, EUROPE) started. Both trials are being carried out between the French and German MDS study groups and DACOTA has been extended to Italy. Challenges associated with EMSCO’s initial activities were identified on four different levels and included:

a) language barriers: impact on smooth communication, risk for delays due to necessary translation of trial documents

b) regulatory differences: procedures cannot be transferred 1:1 from one nation to another as local requirements differ (e.g. one central ethics committee in France vs. one ethics committee per centre in Germany)

c) budgetary issues: costs for trial related activities can differ between countries - complicated budgetary negotiations and allocations

d) tax issues between countries: are to be carefully excluded when planning cross-border trials, especially when both for-profit and non-profit organizations are involved (different taxation).

Conclusions
Cross-border collaboration is a challenging task. This calls for experienced and dynamic multinational teams and close collaboration. EMSCO has made promising first steps in this direction and is about to establish itself as an important European MDS platform.

References
1. https://clinicaltrials.gov/

[58] Development of a DNA-Based Targeted Assay for Interrogation of Chromosomal Deletions

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Introduction
The loss of chromosomal material is frequent among different clonal hematopoetic disorders; one striking example is the deletion of chromosome 5q, often observed in myelodysplastic syndrome (MDS). The detection and appropriate monitoring of these deletions have become important elements of clinical routine diagnostics. Several methods are available and are partly implemented in standard diagnostic procedures such as classical metaphase cytogenetics, FISH, SNP-Array or CGH. All these methods harbour specific disadvantages as they depend on (viable) cells or are very costly, hence we here present a PCR-based assay for paradigmatic targeted interrogation of deletion 5q in MDS. This is based on the normalized quantification of allele ratios from heterozygous microsatellite loci within the commonly deleted region (CDS).

Methods
Based on the UniSTS-database (NCBI) several fluorochrome-labelleed PCR amplicons were designed surrounding microsatellites within the CDS of 5q31-3q33. Multiplex-PCR were performed with 12 primer sets each covering a microsatellite loci.Fragment analysis was carried out on an ABI3130 with internal size standards. Allele calling and peak quantification was performed using ABI Genemapper®. PCR-stutter correction was derived from multiple quantifications of homoyzogous loci from non-deleted samples. Based on the peak-ratio of heterozygous loci, the allelic skewing was determined and normalized to mesenchymal stromal cells as germline control, which were then averaged over all heterozygous loci and transformed into proportion of deleted cells. For interphase-FISH probes targeting Chr5q33 were used. DNA was isolated from various cellular bone marrow or blood compartments from MDS/AML patients with cytogenetically confirmed deletion 5q.

Results
So far a total of n=559 various samples from n=67 patient were quantified using this assay. The mean number of informative loci for a patient was 7. Average standard deviation among n=326 samples analysed as duplicates was 0.86%, when 10ng genomic DNA was used as input. Quantitative correlation of the proportion of deleted cells via side-by-side comparison using FISH or extracted DNA from the very same sample was carried out in n=9 samples resulting in r²=0.93. A serial dilution experiment of deleted and non-deleted DNA gave concordant results with r²=0.96. No microsatellite instability was observed in our cohort of MDS/AML cases. Thus we could quantify the clonal burden for del5q in all samples, where small amounts of DNA could be isolated without prior amplification.

Conclusions
This microsatellite assay offers a mean to obtain quantitative data for basically any intended large scale chromosomal deletion which contains microsatellites. We show a good correlation with established methods exemplarily for Chr5q deletion. Furthermore this test can likewise quantify acquired copy-number neutral loss of heterozygosity. Even if no germline control is available we have developed a robust method to compute allelic imbalances allowing us to quantify clonal burden. This test requires minimum amount of DNA input (tested until 1ng), as it can be derived from archival DNA especially when no intact cells for FISH are available and due to the fragment size it can also be carried out from FFPE-material. The turn-over time is 2 days with a hands-on-time of 2h and it is implemented into 96well format. Therefore this assay bridges a gap for accurate targeted quantification of chromosomal deletions of greater sample batches especially if only limited material is available.
[59] Impact of Iron Overload and Iron Chelators on Osteoblast Functions

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Objectives and background
Iron overload is very common in myelodysplastic syndromes (MDS) – both due to blood transfusions and inefficient erythropoiesis. At the same time, iron excess has been implicated in the development of osteoporosis in hematologic diseases with increased iron content. However, the impact of iron overload on the stromal cell functions and the underlying mechanisms are poorly defined. Several publications point to a role of Wnt signalling in bone remodelling. Our aims are, therefore, to clarify the effects of iron overload and iron deficiency on the main osteoblastic functions, such as proliferation and differentiation, as well as to define a role of Wnt5a signalling pathway in these processes.

Methods
Primary human mesenchymal stromal cells (hMSC) were collected from healthy donors (aged 22-49 years, mixed gender) and differentiated using ascorbate phosphate, b-glycerophosphate and dexamethasone. Murine mesenchymal stromal cells (mMSC) were isolated from wild-type mice by flushing out bone marrow and differentiating towards osteoblasts using an osteogenic cocktail. Cells were treated with FeCl3 (5-50 µM) and an iron chelator deferoxamine (DFO, 5-50 µM). The cell viability was measured using Cell Titer Blue Assay (Promega, USA). The mineralization capacity was determined using Alizarin Red staining, eluted with cetylpyridinium chloride, and quantified using a spectrophotometer. Osteocalcin, Runx2, alkaline phosphatase (ALP), Wnt5a and FZD5 expression was determined by real-time PCR and normalized to β-actin. ALP activity was measured by the enzymatic conversion of p-nitrophenyl phosphate. A Wnt signalling array from SA Bioscience of FeCl3- and DFO-treated murine osteoblasts was performed.

Results
Cell viability of mMSC was not affected after iron treatment, whereas higher concentrations of DFO (>25 µM) reduced cell viability by 20%. hMSC were similarly sensitive to DFO, but also showed a reduced viability in the presence of more than 5 µM iron. The mMSC differentiation potential towards osteoblasts was dose-dependently reduced after iron treatment, reaching a maximum suppression at 50 µM (70%). The expression of osteoblastic markers, such as Runx2, osteocalcin and ALP, as well as ALP activity, were significantly decreased after iron treatment in mMSC, whereas only high iron concentration (50 µM) could induce the same effects in hMSC. In contrast, iron chelation using DFO increased osteogenic differentiation by up to two-fold in murine and human MSC. This was accompanied by a significantly increased expression of osteoblast markers including Runx2, ALP, and ALP activity. Interestingly, a significant 3- to 5-fold increase in Wnt5a expression was observed in hMSC and mMSC after DFO treatment. The expression of its cognate receptor FZD5 was not altered by DFO treatment. However, the Wnt array revealed a significant downregulation of the Wnt inhibitors Sfrp1 and Sfrp2 after DFO treatment.

Conclusions
Our results demonstrate that iron inhibits MSC viability and differentiation, while the iron chelator DFO increases osteogenic differentiation and leads to a strong activation of Wnt5a signalling. Whether the effects of DFO on Wnt signalling are dependent on iron chelation or whether other mechanisms play a role requires further clarification.

[60] The effect of combination of dimethylcelecoxib and imatinib on NF-kB expression in colon cancer cells

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Objectives and background
2,5-dimethyl-celecoxib (DMC), a close structural analogue of celecoxib without cyclooxygenase inhibitory side effects, mimics all of the numerous anti tumor effects of celecoxib in vitro and in vivo. In colorectal cancer, NF-κB has a role in progressive through expressions of various gene targets involved in cell proliferation, angiogenesis, and metastasis. In the present study, we examine the synergistic actions of c-kit inhibitor imatinib and DMC or celecoxib on NF-κB expression.

Methods
Human colon cancer HT-29 cells were treated with imatinib and celecoxib/DMC alone or in combination for 24 hours. Total cellular RNA was isolated using a RNX-plus solution. Then these total RNAs were reverse-transcribed and the resultant cDNA mixtures were subjected to the amplification by real time PCR. mRNA expression of NF-κB was evaluated by Q-RT-PCR and data were analysed with ddct method.

Results
We observed significant (p<0.05) decreased expression of NF-κB when cells were exposed to low doses of dimethyl celecoxib (12 µM) and imatinib (3.5 µM) combination compared to control group. Also NF-κB expression decreased in imatinib (3.5 µM) and celecoxib (15 µM) combination, but it was not significant.

Conclusions
Thus we conclude that imatinib and DMC combination inhibits activation of NF-κB, which regulates many downstream pathways that are essential for cell survival. Collectively, our data provide a novel molecular mechanism for the antitumor activity of DMC. This raises the intriguing prospect that the use of dimethylcelecoxib could be a highly promising strategy for colon cancer chemoprevention while minimizing undesired side effects.
[61] Comparative effects of imatinib in combination with celecoxib or 2,5-dimethyl celecoxib on proliferation of Human Colorectal adenocarcinoma Cell Lines

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Objectives and background
Colon cancer, the most common solid epithelial malignancy is c-kit positive and responsive to the specific tyrosine kinase inhibitor imatinib. A novel compound, 2,5-dimethyl-celecoxib (DMC), a close structural analogue of celecoxib, does not have cyclooxygenase inhibiting properties but mimics the anti tumor effects of celecoxib. The purpose of this study was to compare the effects of imatinib-celecoxib and imatinib-DMC on the proliferation of colorectal cancer cells in vitro.

Methods
HT-29 cells were treated with imatinb and celecoxib or DMC alone or in combination. Proliferation inhibition of cells was assessed by Tetrazolium-based cell line proliferation assay (MTT assay) after 24 hours. IC50 values were determined using CompuSyn software. To determine the interaction between the drugs, the combination index (CI) was calculated using the Chou Talalay method.

Results
The results showed that imatinib, celecoxib and DMC decreased tumor cell proliferation with IC50 values of 7.62, 30.41 and 23.45 µmol/L, respectively. Isobologram analyses revealed strongly synergistic drug interactions, with combination index of 0.664 for celecoxib/imatinib combination and 0.370 for dimehylcelecoxib/imatinib combination. However the concentration of DMC (12µM) was lower than celecoxib (15µM), but synergistic effects of its combination with imatinib was 1.79 fold greater than the first combination.

Conclusions
Taken together, the results demonstrated a strong synergy between the actions of imatinib and celecoxib or DMC in growth inhibition of human colon cancer cells. The synergic effects of DMC and imatinib combination were much stronger than that by combination of celecoxib and imatinib. However, the molecular mechanisms underlying their combined actions are not well understood, celecoxib might exert its effects by COX-2 dependent and independent mechanisms. Therefore it would be beneficial to further evaluate DMC's potential as a non-cecoxib alternative to celecoxib for anticancer application.

[62] NOSTRuM – New therapeutic Options for Solid and hemaTological Rare Malignancies

Boosting and sharing integrated Next Generation Sequencing, in vitro and in vivo approaches for targeted personalized therapy of rare tumors: from bench to bedside

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Background and objectives
Next generation sequencing studies have recently shown that multiple genetic hits participate to tumor development and relapse by altering crucial biological processes. The spectrum of genetic lesions, along with their functional consequences, determines the high level of heterogeneity observed at the inter-individual level, being reflected by different clinical outcomes and responses to treatment among patients affected by the same tumor. These evidence indicate that each tumor represents an individual rare entity and accurate molecular characterization is needed to guide clinical decisions. Previous experience with the European LeukemiaNet (ELN), an EU-funded organization of physicians, scientists and patients with interest in leukemia, paved the way for building a network aiming at strengthening and developing scientific and technological excellence in research and therapy of leukemia.

Methods
The present project aims at improving the treatment and understanding of rare solid and hematological malignancies in Europe and at spreading excellence, by integrating the leading national leukemia networks and their interdisciplinary partner groups in Europe. This objective will be pursued through the characterization of common pathways aberrantly activated or silenced across different rare human cancers, that can become molecular targets, and the design of personalized therapeutic protocols for rare tumors, whose management is further challenged by individual heterogeneity. In the wake of ELN mission and by expanding the ELN platform, the project will integrate diagnostics, translational and clinical research. The structure will resemble the ELN organization, which includes specific diseases and common tasks with a number
of working packages (e.g. Next Generation Sequencing (NGS), gene expression profiling, diagnostics, cytogenetics). Primary samples will be analyzed by NGS technologies (whole exome sequencing, RNA sequencing) to design a map of driver and passenger genetic abnormalities and connect them to transcriptional alterations across tumors. Potential therapeutic targets will be identified and orphan drugs acting on the specific pathways will be selected. The anti-tumor activity of the pharmacological compounds will be tested through in vitro and in vivo approaches to define the most promising ones. Clinical trials will be designed which allow the combination of conventional chemotherapeutic agents with target therapies selected on a molecular base.

Expected outcome
The results of the proposed study, that will be presented to the European Commission for funding (Horizon 2020, Personalising health and care, PHC-14-2015: New therapies for rare diseases), are expected to have a major impact on the clinical practice and will rapidly improve the management of tumor still representing a major challenge for medicine.
This activity is an official meeting of the ELN LeukemiaNet®

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