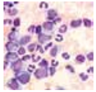
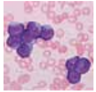
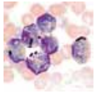
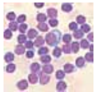
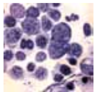
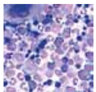

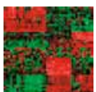
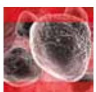

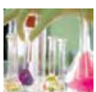
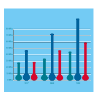

European LeukemiaNet

INFORMATION LETTER OCTOBER 2010

ABSTRACTS

ELN Frontiers Meeting

New benchmarks in leukemia:

Focus on CML, AML and MDS

Chairs: Günther Gastl, Michele Baccarani, Rüdiger Hehlmann

22 - 24 October 2010, Vienna, Austria

Dear Colleagues

ELN Frontiers is an annual educational program of the EUTOS (European Treatment and Outcome Study for CML) project, a collaboration between the European LeukemiaNet and Novartis Oncology Europe. ELN Frontiers started in 2006 and is held in major cities in Europe (Venice 2006, Budapest 2007, Cannes 2008, Barcelona 2009). The educational event attracts each year over 500 international leukemia experts.

This year's event takes place in Vienna, a city often described as Europe's cultural capital with unique charm and flair, famous for its history, architecture, culture and music. The meeting is hosted by Prof. Gastl, who is also President of the Austrian Society of Hematology/Oncology.

In its last contractual year the EUTOS project emphasises major outcomes also with respect to other leukemia entities within the European LeukemiaNet. This is reflected in the title of the meeting: New benchmarks in leukemia: Focus on CML, AML and MDS.

Another new feature within the program is the call for abstracts. 21 exciting topics were submitted, ten in the field of CML, seven in MDS, three in AML and one in the field of other myeloproliferative neoplasms.
Topics range from treatment situations in individual countries, treatment results, diagnostic studies like on expression, FISH analysis and follow up of mutations influencing response rates, or characterisation of disease stage. Three outstanding abstracts were awarded for oral presentations.

The program addresses clinically active health care professionals and scientists actively involved in leukemia research. The abstract book will give you an overview of the posters and lectures that will be presented at the conference.

We are looking forward to this important event in leukemia and to the continuation of the ELN Frontiers meetings in the future.

Yours sincerely

G. Gastl, M. Baccarani and R. Hehlmann

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[1] Follow-up of childhood CML with monitoring the BCR-ABL gene expression in peripheral blood -case report-

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Background and objectives

Chronic myelogenous leukemia (CML), a myeloproliferative disease characterised by the presence of the Philadelphia chromosome or the BCR-ABL fusion oncogene, accounts for 15-20% of adult leukemias, but is rare in children (2%). Imatinib has changed the outcome of CML, being the first molecular targeted cancer therapy, by blocking the BCR-ABL tyrosine-kinases in their signalling pathways for proliferation. However, the only curative treatment is the allogeneic bone marrow transplantation. The objective of this paper is to present the results of imatinib therapy combined with RT-PCR monitoring of the BCR-ABL gene expression in peripheral blood of a 16-year old CML patient, over a period of 18 months.

Case report

A 16-year old boy presented with fatigue, abdominal pain and pallor. On physical examination he was pale and no lymph nodes were palpable. Arterial blood pressure: 118/68 Hgmm, heart rate: 68/min, in his abdomen a giant splenomegaly is palpable at 20 cm below the costal margin and a moderate hepatomegaly. His WBC count was 424000/mm^3, Hgb:10.4 g%, Htc: 25%, platelets: 348000/mm^3, reticulocytes: 46%. The peripheral blood picture: myeloblast 1%, promyelocyte 2%, myelocyte 2%, metamyelocyte 12%, band 9%, segmented 36%, eosinophil 2%, basophil 5%, monocyte 1%, lymphocyte 1%, erythroblasts 2/100 leukocytes, macrocytes of 9µ. The bone marrow is hypercellular with 1% myeloblasts, a hyperplastic myeloid line and basophilia. The leukocyte alkaline phosphatase was 0. Quantitative real-time PCR examination from the peripheral blood showed the expression of BCR-ABL gene in comparison with the control ABL gene in 140%. Blood chemistry showed a high lactate dehydrogenase (LDH) level (2105 U/l). Cytogenetic examination of the bone marrow revealed 35% Ph+ cells. The ultasonographically measured size of the liver was 188/85 mm and of the spleen was 280/116 mm. Eye fundus examination showed hyperemic papillae, peripapillar hemorrhage and dilated, sinuous retinal vessels. The chronic phase CML diagnosis was made with an intermediate Sokal and Hasford score (0.94 and 1142 respectively). Imatinib treatment at the dose of 400 mg/day was started. The details of the 18 months follow-up are shown in table nr. 1.

Major cytogenetic response was achieved after 3 months of daily 400 mg imatinib therapy, whereas major molecular response in 12 months time, with a higher than 3 log reduction in BCR-ABL gene expression level. No side effects were noticed due to imatinib. Family members were examined for human leukocyte antigen compatibility, regarding a further bone marrow transplant, but no compatible donor was found.

Conclusion

We presented the case of a 16-year old male patient with chronic phase CML on imatinib 400 mg/day therapy, who has been monitored by molecular biology from peripheral blood and bone marrow cytogenetics over a period of 18 months as recommended by the European LeukemiaNet.

	Diagnosis	3 months	6 months	12 months	18 months
WBC/mm^3	424000	3030	3920	3250	5100
Hgb (g/dl)	10,7	11,3	11,5	12,7	11,8
Htc (%)	26,6	35,5	35,2	38,9	36
PLT/mm^3	348000	78000	100000	145000	190000
Basophils in blood (%)	5	2	1	0,3	0,8
Blasts in BM (%)	1			0,5	
Ultrasonographic spleen size (mm)	280/116	190/51,2	157/50,7	125/34,5	
Cytogenetic exam BM	Ph^- cells 35%		Ph^- cells 0%	Ph^- cells 0%	
QRT-PCR blood: BCR-ABL gene expression (%)	140	1,41		0,0004	
Sokal score	0,94				
Hasford score	1142				
Response to treatment		No HR	MCR	MMR	

Table: A 18 months monitoring of a 17-year old CML patient treated with imatinib 400 mg/die
HR- hematological response, MMR- major molecular response, MCR- major cytogenetic response

CML

[2] 10-Year survival of patients with late chronic phase Ph-positive chronic myeloid leukemia receiving tyrosine kinase inhibitors therapy after interferon failure

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Background
Imatinib (IM), a potent, selective BCR-ABL inhibitor, provides an effective treatment for chronic myeloid leukemia (CML). Objectives: To evaluate the efficacy of tyrosine kinase Inhibitors (TKI) and 10-year survival of patients (pts) in late chronic phase (L-CP) CML by the database called Hematological Research Center Register (HRCR is a part of the National Russian CML Register).

Patients and treatment
This non-randomised, open-labeled trial recruited patients from July 2000- September 2001 till July 2010. In total, 79 pts with L-CP CML resistant/intolerant to IFN- failure enrolled. Median (Med.) age was 39 (15- 64) yrs, sex ratio (M:F) 41:38. Med. time from CML diagnosis to IM treatment was 35.1 (3 - 157) mo and it was equate of Med. time pretreatment with IFN. Sokal risk stratification: 63% Low/19% Intermediate/18% High. Initial IM dose was 400 mg/day. Med. follow-up on IM treatment at the time of analysis (July 2010) was 80 mo (2.4-118). Med. duration of TKI-2 treatment was 31 (2-51) mo. More than three TKI received: 4(5%) pts. Med. duration of the disease from diagnosis till analysis was 134 (13-259) mo; 55(70%) pts are alive for more than 10 yrs after CML diagnosis, 8 of them with longevity of CML for more than 15 years.

Results
59 of 79 pts (75%) are alive as of July 2010. CHR on IM was achieved in 75 (94,5%) pts with a Med. time to response of 3 (1-20) mo. Of 75 pts, 33 (44%) lost CHR; 15 (19%) pts returned to CHR on IM and 6 (7.6%) pts with TKI-2. The cumulative incidence of CCyR was 54.4% (43 pts) with a Med. CCyR at 9 mo (5-53). Acquired Cy resistance- in 50% of pts. CCyR was reached again on IM in 27 (34%) pts (still on IM) and 8 (10%) pts- firstly with TKI-2. For the entire period of observation, 64% obtained CCyR, 71% MCyR. According to the ELN criteria from 2009, only 9 of 43 pts (21%) with L-CP achieved CCyR at 12 mo of IM therapy. Stable CCyR was observed in 22 of 43 pts (51%). 34 (43%) pts continued on IM therapy with CCyR. 46 (58%) pts discontinued IM therapy. 29 (35%) pts switched to TKI-2. 20 (25%) pts died (16 pts with AP/BC, 2 pts with brain hemorrhage, 1 pt with generalised infection, 1 pt with hepatocellular carcinoma). CML-related cases of death were at the same level for first 6 yrs (2.5%) and decreased with age. Importantly, the 9-yr period of IM therapy revealed only 2 (2.5%) cases of secondary malignances (1 pt alive with CMolR). OS with CCyR and MCyR were 95% and 94% v. 50% and 44% without CyR, accordingly ($p<0.0001$). Two pts with MCyR, one- with CCyR and one without MCyR died of non-CML-reasons. Estimated 10-yr OS of pts receiving TKIs after IFN-failure is 75%, progression-free survival is 81%. OS by Sokal risk groups at 10 yrs of treatment were 86% Low/ 67% Intermediate/ 50% High, accordingly ($p=0.0094$).

Conclusions
TKIs resulted in significant improvement of OS and prognosis in CML- pts resistant or intolerant to IFN. Data for analysis received by the HRC Register and will observe in the future.

CML

[3] Current situation in treatment of chronic myeloid leukemia in Estonia

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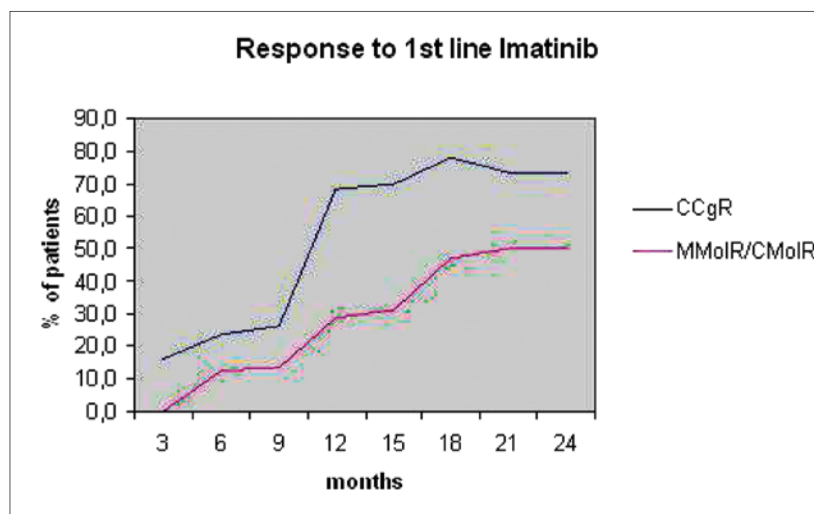
Objectives and background
The objective of the study is to present an overview of treatment of CML in Estonia. Tyrosine kinase inhibitors (TKI) have been available as first-line therapy for CML since July 1, 2006. From October 2002, until June 2006, imatinib could be used for patients with progressive disease or intolerance to interferon. At the beginning of January 2010, there were altogether 75 CML patients under regular control and treatment in two hematology centres in Estonia, diagnosed in 1987-2009: 40 women and 35 men with median age of 53,1 years (range 17-78) at diagnosis. 61 patients presented in chronic phase (CHP), 11 in accelerated phase (AP) and 3 in lymphoblastic crisis (BC).

Methods
This study was conducted on clinical records.

Results and statistical significance
34 patients (32 CHP, 1 AP, 1 BC) received imatinib as first-line therapy, 9 of them for less than 9 months by January 2010, and therefore not assessed. Among those in CHP, optimal response was seen in 11 patients (45,8%) and suboptimal response in 7 patients (29%). Only hematological response was seen in 3 patients and no reponse at all in 2 patients. The patient in AP had a CCgR at 12 months, but due to non-hematological adverse effects continued treatment with nilotinib.
Imatinib as ≥2nd line therapy was used in 39 patients. Among those in CHP (n=24) optimal response was seen in 10 patients (43,4%), suboptimal response in 5 patients (21,7%). Only hematological response was seen in 5 patients. In the AP group (n=11) optimal and suboptimal responses were seen in 3 patients each (27,3%). In the BC group (n=4), 1 patient gained 2nd-CP and was allotransplanted, 2 patients had a hematological response and continued therapy with nilotinib, one of them was later allo-transplanted; 1 patient had CCyR at 12 months, CMolR at 18 months, but at 30 months progressed to 2nd-BC.
Altogether, 13 patients were switched to a 2nd-generation TKI due to suboptimal response, treatment failure or intolerance to imatinib. There was 1 optimal response, 2 suboptimal responses, 4 hematological responses. In 5 cases the treatment time was too short to assess the effect.



Conclusions
Mostly, the treatment results are comparable to those published in the literature, yet we saw a lower rate of MMolR (Figure 1). However, we found a lower rate of suboptimal response at 18 months. Our data also show the similar rate of optimal response to 1st and 2nd line imatinib therapy in CP group.

CML

[4] Monitoring cytogenetic and molecular responses in CML: An experience of 5 years

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Background and Objectives
Chronic Myeloid Leukemia (CML) is a myeloproliferative disease characterised by the presence of the Philadelphia chromosome (Ph), which results from a reciprocal translocation between chromosomes 9 (ABL gene) and 22 (BCR gene) leading to the formation of the BCR-ABL fusion gene. The expression of the fusion transcript leads to deregulation of the tyrosine kinase activity of the protein encoded by the ABL gene. The development of the BCR-ABL tyrosine kinase inhibitor imatinib mesylate has revolutionised the treatment of CML. However, some patients don't respond or became resistant to the treatment. The main mechanism of resistance so far identified is the acquisition of point mutations in the kinase domain of the BCR-ABL fusion gene.
Our aim was to perform a cytogenetic and molecular follow-up of new patients diagnosed with CML in the last 5 years.

Methods
Since 2005, we evaluated 63 new cases of CML that started treatment with imatinib. The presence of t(9;22) was confirmed by conventional cytogenetics and/ or by FISH, and the fusion transcript was detected and characterised by RT-PCR. To monitor the cytogenetic response, we calculated the percentage of Ph+ metaphases in at least 20 karyotypes from bone marrow samples. To monitor the molecular response, the number of transcripts was quantified by RQ-PCR. To investigate mutations in the kinase domain of the fusion protein, the region corresponding to the ABL gene in the fusion BCR-ABL was amplified by RT-PCR and exons 4-9 were sequenced. The sequences obtained were compared with a reference sequence (GenBank NM_005157.3).

Results
In the 63 patients studied, 45 achieved CCR, median of 12 months, 2 relapsed before reaching the RCC, 1 didn't have any response and 15 achieved partial or minimal cytogenetic response or had no cytogenetic evaluation. 35 patients had an MMR, median of 14 months, 14 achieved Complete Molecular Response (CMR) and 27 had insufficient follow-up or sub-optimal response. Of the patients who achieved CCR, 11 did not have MMR.
From a total of 8 patients that failed complete cytogenetic response (CCR) or Major Molecular Response (MMR), we have identified a mutation (E255K) in one patient. Following modification of treatment to a second-line tyrosine kinase inhibitor the mutation was no longer detected.

Conclusions
Monitoring cytogenetic and molecular responses in patients with CML, as well as the identification of mutations in the kinase domain of BCR-ABL fusion gene, is of major importance to optimise the therapeutic strategy.

CML

[5] Retrospective analysis of correlation between achieving CCyR and prognostic score according to Sokal and Hasford

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Background and Objectives
Most patients achieved complete cytogenetic response (CCyR) or partial cytogenetic response (PCyR) after 12 months on imatinib first-line treatment. The prognostic classifications proposed by Sokal and Hasford were invented for chronic myeloid leukemia patients treated with hydroxyurea and interferon-alpha, but remain valid for imatinib treatment. In the IRIS study, at 12 months, the CCyR rates were 78%, 68% and 51% for low, intermediate and high–risk patients, respectively according to Sokal and the differences between the groups were significant (p<0.002). We retrospectively analyzed whether achieving CCyR or PCyR correlates to prognostic score, according to Sokal and Hasford.

Patients
167 patients with newly diagnosed CML, treated with imatinib in Poland between 2004-2007 were analyzed. All analyzed patients at 12 months of imatinib therapy achieved a CCyR or PCyR. Patients with more than 35% Ph positive metaphases were excluded from the study due to the low number of patients in our database. Median age according to Sokal was 44, 55 and 52 years old for low, intermediate and high–risk patients, respectively. Sokal prognostic score distribution rate were 40%, 38% and 22% for low, intermediate and high–risk patients, respectively. Hasford score was 42%, 51% and 7% for low, intermediate and high–risk patients, respectively.

Results
Percentage of patients with CCyR at 12 months according to Sokal score were 74% , 77% and 71% and with PCyR were 26%, 23% and 29% for low, intermediate and high–risk patients. There were no differences in the probability of achieving CCyR or PCyR between low, intermediate and high–risk groups according to Sokal (chi square analysis).
The same group of patients was analyzed according to their Hasford score. The percentage of patients who achieved CCyR at 12 months was 79%, 78% and 58% for low, intermediate and high–risk patients, respectively. In statistical analysis (chi square), there was statistically significant difference in the probability of achieving CCyR in intermediate and high (p=0.0011) and low and intermediate (p=0.012) risk groups according to Hasford. A similar result was found for PCyR between intermediate and high (p=0.0044) and low and high (p=0.172) risk groups. No statistically significant differences were found for low and intermediate risk groups according to the Hasford prognostic score.

Conclusion
The probability of achieving a CCyR or PCyR is much lower in the high-risk group according to Hasford, in comparison to the low or intermediate risk group. Such a correlation was not found in the risk groups according to Sokal prognostic score.

CML

[6] Activity of EMD Serono-AS703569, a multi-kinase inhibitor of BCR-ABL and Aurora Kinases in BCR-ABL transformed cells, is dependent on Aurora B inhibition, and is not affected by the presence of the highly imatinib resistant BCR-ABL mutation T315I

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The tyrosine kinase inhibitor imatinib is the gold standard in conventional treatment of CML. However, the emergence of resistance to IM remains a major problem. Alternative therapeutic strategies of IM-resistant BCR-ABL positive leukemias are urgently needed. One promising target for anti-cancer therapeutics is represented by the Aurora kinase family. These serine/threonine kinases are involved in regulating multiple steps of mitosis, including formation of bipolar spindle, chromosome alignment, spindle checkpoint function and cytokinesis.

We report on studies accomplished with a small molecule inhibitor AS703569 (Merck Serono), which targets BCR-ABL and Aurora kinases A-C. We could show that AS703569 exhibited strong antiproliferative and pro-apoptotic activity against murine BaF3- cells ectopically expressing wildtype (wt) or IM-resistant BCR-ABL mutants, including those harbouring the strongly resistant T315I mutation. This effect was already observed at rather low-AS703569 concentrations, at which Aurora, but not the BCR-ABL kinase was inhibited. Furthermore, in cell cycle analysis we observed cells with a large 4N peak and DNA content more than 4N, indicating extensive polyploidisation, a consequence of continued cell cycle progression in the absence of cell division. Recent studies have revealed that this phenotype is based on suppression of Aurora B kinase activity, indicating that Aurora B inhibition is the major effect of AS703569 in BCR-ABL positive cells. To confirm this assumption we designed pBabe-puro based retroviruses encoding different point mutations in the Aurora B ATP binding site, which should lead to resistance against AS703569. By this strategy we were able to identify an AS703569 resistant mutant (Aurora B G216V). This mutant shows significant resistance in vitro and in vivo and is able to augment the antiproliferative capacity of AS703569 in BCR-ABL positive cells. Taken together, our data demonstrate that anti-proliferative effects of AS703569 in BCR-ABL positive cells are primarily mediated by functional inhibition of Aurora kinases, especially of Aurora kinase B. As these kinases are clearly implicated in tumorigenesis, they will become high potential therapeutic targets for anti-cancer therapy.

CML

[7] Successful treatment of mixed-cell CML blast crisis without chemotherapy

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Objectives and background
The treatment of CML in the last decade has undergone substantial changes in methods and results. Introduction of new innovative target-directed drugs and more thorough techniques of disease control have improved outcome in CML patients. However, this drug is not a magic bullet, even for with long-term disease control. Prevention of disease progression, and its treatment still remains one of the main objectives in CML management.

We present a case report of leukemic transformation with a mixed cell phenotype after a 9-year chronic phase. The patient was male, born in 1948, and diagnosed with CML in 2001, with leukocytosis and 100% Ph+ cells in the bone marrow with the b3a2 type of transcript. There were no siblings. Treatment was started with hydroxyurea. From April to October 2004, the patient had received peg-interferon. Treatment was stopped due to fatigue and grade IV TTH reduction. Since the December 2004, the patient was treated with imatinib, and had a complete hematological response in 3 months and partial and complete cytogenetic response at 6 and 12 months of imatinib treatment. Major molecular response was not achieved. In control CBC in February 2010, 8% blasts were revealed. In examination there were 95% bone marrow blasts with CD7+,CD13+,CD20+,CD33+,CD34+,HLA-DR+,cyCD22+TdT+,MPO- immunophenotype as partly presented in fig.1.

Chromosome banding analysis demonstrated 83-85, XXXYYY,t(9;22)(q34;q11),+der (22)t(9;22)[16]/46, XY [4] karyotype. By EGIL scores, condition was verified as mixed-cell phenotype CML blast crisis. Considering age and blast immunophenotype, induction regimen consisted of 8 weekly infusions of rituximab 375 mg/m^2 and dasatinib 140 mg daily.

Results
After 1 month of therapy complete remission was reached with 37-38, XY, del(6)(q24),-10,-15,-18[4]/46,XY[16] in cytogenetic. There were no significant toxicities except persisting grade II enteropathy, which was the reason for dasatinib dose reduction to 100 mg daily after 2 months of therapy. Complete cytogenetic and molecular responses were verified after 3 months of treatment, which was confirmed in the 6 month follow-up assessment. Nevertheless, there was residual population of blasts with initial phenotype by flow cytometry in 3 and 6 months of treatment (0.0005% and 0.011% accordingly).

Conclusions
Despite of substantial improvements in management, disease transformation is not fully prevented. The usage of new target-directed agents could be very effective in resolution of CML blast crisis, but there is a need to develop a strategy for long-term surveillance.

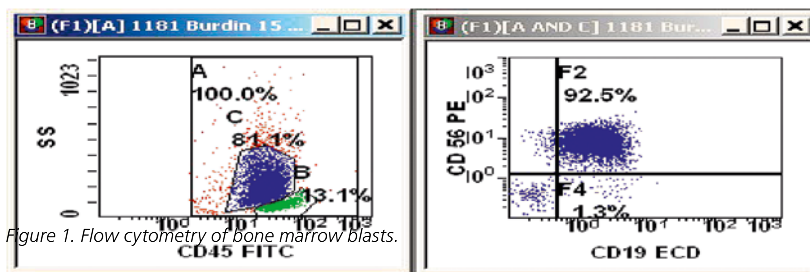

Figure 1. Flow cytometry of bone marrow blasts.

CML

[8] Clonal chromosomal abnormalities in Ph-negative cells of chronic myeloid leukemia patients, treated with tyrosine kinase inhibitors

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Objectives and background

The practical significance of chromosomal abnormalities (CA) in Ph-negative cells during CML treatment with tyrosine kinase inhibitors (TKI) is still undefined. The aim of this study was to estimate the influence of CA on CML clinical course during treatment with 1st- and 2nd-generation TKI.

Methods

Four hundred forty three CML patients were treated with TKI in the National Research Center for Hematology in 2000-2010; in 50 of them we have found CA in Ph-positive, in 10 - in Ph-negative and in 3 - both in Ph-positive and in Ph-negative cells. This study analyzes 13 CML patients with CA in Ph-negative cells (7 males and 6 females, age 25-69, median age - 46 when CA were revealed). At CML diagnosis, 11 patients had CP (Sokal low-risk in 4, intermediate-risk in 5 and high-risk group in 2 of them), 2 - AP, remaining at the moment of CA diagnosis and data analysis. The disease duration was 46-201 months (median - 85), all the patients were pretreated (2-100 months before TKI, median - 22 months). All patients received imatinib 23 - 107 months (median - 55). The patients with imatinib failure received nilotinib (n=1, 42 months), dasatinib (n=2, 52 and 4 months), bosutinib (n=1, 19 months) and sequentially nilotinib and dasatinib (n=1, 3 and 34 months). The cytogenetic response was estimated by G-banding and FISH.

Results and statistical significance

CA in Ph-negative cells were found in 3% patients (n=13), treated with TKI; all of them had +8, in addition 1 patient had –7, 2 - abnormalities of Y, 1 - +X. They were revealed only after achieving MCR (at retrospective examination by FISH, it could be found even among 18% Ph-negative cells); in 10 patients - during high-dose imatinib treatment, in 3 - at treatment with 2nd-generation TKI. They were revealed after 12-63 months on 2nd-generation TKI therapy (median - 27 months).
The response to TKI treatment was rather late: partial cytogenetic response (PCyR) was achieved after 6-63 months (median - 21), complete CyR (CCyR) - after 12-63 months (median - 39); in majority of patients the Ph-positive clone persisted for some time. Then the response stabilized: 1 patient (8%) achieved PCyR (that was subsequently lost), 12 (92%) has achieved CCyR, that is still remaining. All patients are alive. Two patients have constant abnormalities in Ph-negative cells and in 7 patients they were found only at 1 or 2 analyses.
The comprehensive morphological, cytochemical, histological bone marrow analyses and biochemical markers of dyserythropoiesis didn't reveal any signs of myelodysplasia.

Conclusion

The additional chromosomal abnormalities in Ph-negative cells of CML patients are rare; in our study they were found in 3% of patients, all of which had trisomy 8. At first time they could be found when sufficient number of Ph-negative cells appeared in bone marrow (at MCR achievement). These clones could be only once or repeatedly, they persisted for a long time or exhausted. Meanwhile, 92% of patients have achieved stable complete CR with high-dose imatinib or 2nd-generation TKI treatment, despite its late achievement. Though trisomy 8 or monosomy 7 is traditionally regarded as myelodysplasia, there were no signs of it or acute leukemia transformation. These abnormalities need further investigation with a larger group of patients.

CML

[9] Identification of BCR-ABL/ABL ratio increase that corresponds to BCR-ABL mutation in chronic myeloid leukemia patients

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Background

Currently there is no consensus in the definition of what level of BCR-ABL/ABL ratio increase predicts presence of kinase domain (KD) mutations. Several research groups use relatively low cut-off levels equal to 2.0- and 2.6-fold (S. Branford et al., 2004, R. Press et al., 2009, respectively), that are close to the discrimination ability of real–time quantitative PCR (RQ-PCR) method. Alternatively, an NCCN guideline recommends beginning of mutation screening in case of 10-fold or greater elevation of BCR-ABL/ABL ratio.

Objective

To define a threshold level of BCR-ABL/ABL increase that predicts presence of BCR-ABL mutations.

Methods

Among 531 CML patients on imatinib (IM), both newly diagnosed and pretreated with interferon-α, 47 had BCR-ABL mutation detection was performed. These were patients with suboptimal response or treatment failure according to the European LeukemiaNet criteria. Quantitative measurement of BCR-ABL/ABL transcript ratio by RQ-PCR was done every 3-6 months. A major molecular response was defined as a BCR-ABL/ABL transcript level of 0.059% corresponded to a 3-log reduction from the laboratory defined baseline level. Point mutations in the BCR-ABL KD were detected by reverse-transcriptase PCR and direct sequencing. Elevation of BCR-ABL/ABL was calculated by dividing the BCR-ABL/ABL value at the time point (TP) where mutation detection was performed by the BCR-ABL/ABL value at TP prior to mutation screening. Event-free survival (EFS) was defined as the time from beginning IM until any of the following events occurred: loss of complete hematological response, loss of major cytogenetic response, progression to AP/BC, death of any reason. Threshold level was defined by receiver operator characteristics (ROC) curve analysis. Positive and negative predictive values (PPV, NPV), sensitivity, specificity and overall correct prediction (OCP) were calculated. Results. 10 different point mutations of BCR-ABL gene were detected, including 3 in the P-loop, 2 in the IM-binding site, 3 in the A-loop, and 2 mutations outside the KD. None of patients had 2 or more mutations simultaneously. Patients were divided into two groups: with (n=18) and without (n=29) BCR-ABL mutations. Groups did not differ in age, sex distribution, type of BCR-ABL transcript, frequency of cumulative achievement of CHR, CCyR, MMR and level of BCR-ABL/ABL increase (table 1). EFS was higher in the group without KD mutations (0.26±0.19 vs 0) (p=0.017). ROC curve analysis determined that increasing of BCR-ABL/ABL level by 5.5-fold corresponds to 92.9% of NPV. Area under curve was 68% (95% CI 50-95%) (p=0.022). Sensitivity, PPV and OCP were relatively low (40.6%, 40.6%, 56.5%, respectively) while specificity was high (92.9%).

		Patients with KD mutations (n=18)	Patients without KD mutations (n=29)	P
Median age, (range), years		46 (18-70)	44 (18-67)	0.888
Male sex		12	12	0.135
Sokal risk group	Low	7	9	0.698
	Intermediate	5	12	
	High	6	8	
Treatment with Interferon-α prior to IM		3	2	0.279
Median interval from diagnosis to beginning IM treatment (range), months		9.8 (0.5-81.9)	4.0 (0.6-110.1)	0.401
Type of BCR-ABL transcript	e13a2	4	10	0.289
	e14a2	14	19	
Cumulative achievement of CHR		17	28	0.624
Cumulative achievement of CCyR		3	13	0.062
Cumulative achievement of MMR		2	4	0.581
Median elevation of *BCR-ABL/ABL* ratio at the time of mutation detection(range), times		5.38 (0.01-8.50)	7.02 (0.01-13.82)	0.131
Median follow-up from starting IM, months (range)		27.9 (9.6-45.0)	35.6 (9.7-57.8)	0.566
Event-free survival		0	0.26±0.19	0.017

Conclusions

In our series 5.5-fold increase of BCR-ABL/ABL clearly predicts presence of BCR-ABL mutations and indicates the exact time for mutation detection performing in patients with suboptimal response and treatment failure. Nowadays, with availability of primary reference material for BCR-ABL quantification, approved by WHO and successful harmonization of molecular monitoring of CML therapy elevation level that corresponds with mutation presence could also be standardized. Application of international standardized threshold level would help to avoid unnecessary or late mutation tests.

CML

[10] Factors influencing the stability of CCyR in CML-CP patients on imatinib therapy

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Objectives and Background
The aim of this study was to reveal factors, influencing the stability of CCyR in CML chronic phase (CML-CP) patients on imatinib (IM) therapy, in routine clinical practice.

Patients and methods
In a patient database of the St. Petersburg and Leningrad region, there are 235 CML-CP patients, who received IM for at least 12 months. This study included 115 pts according to the following criteria: IM start dosage of 400 mg/day and CCyR which was confirmed by at least 2 consecutive cytogenetic analyses with 0% Ph+ cells. Patients in CCyR with IM therapy interruptions of more than 3 months were censored at the date of the last cytogenetic analysis.

Results
The median time from diagnosis to IM treatment was 7 months (0.1-108 months) and 55 patients began the IM treatment in early CML-CP (≤ 6 months since diagnosis). 64, 38 and 13 patients had low, intermediate and high Sokal scores respectively. The median observation time on IM treatment was 56 months (16-88 months). Overall estimated probability of CCyR loss was 16%, rate 12.1% (14/115). In 10 patients CCyR was lost within major CyR. The estimated overall survival (all causes of death) was 78% (death rate 4.3% (5/115)), and only 1 death was CML-related.
CCyR loss was less frequent in early CML-CP 10% (rate 3.6% (2/55)) than in late CML-CP 21% (20% (12/60)), p=0.032. For a more thorough analysis, patients in late CML-CP were divided in subgroups related to duration of CML before IM initiation: >6 and ≤12 months, >12 and ≤60 and more than 60 months. Probabilities of CCyR loss in these groups were 22% (rate 20% (3/15)), 27% (rate 25.7% (9/35)) and 0% (rate 0% (0/10)), respectively (p<0.05). The median time to CCyR loss was 29.5 months in early CP, 10.3 months in the group 6-12 months before IM, and 14.2 months in the 12-60 months before IM group (p<0.01).
CCyR was lost in 10% (rate 8.9% (7/78) of patients with CCyR obtained within 12 months of IM treatment with a counterpart of 28% (18.9% (7/37)) for late-responders (CCyR after 12 months IM), p=0.02. The further subdivision by the time to CCyR achievement did not reveal any significant differences.
Among patients, who lost CCyR, only 2/14 (14%) patients progressed to blast crisis; one of them was treated with chemotherapy followed by allo-SCT and is still alive in CMR. Another patient was treated by high dose IM with chemotherapy, but died due to progressive disease.

Conclusions
Patients in CML-CP with CCyR have a very good prognosis. There are two factors, which influence the probability of CCyR loss: initiation of IM in early CP and CCyR in the first year of treatment are favorable for good prognosis for CCyR durability.

[11] Integrative prognostic risk score in acute myeloid leukemia with normal karyotype

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** and $ denote equal contribution*

Objectives and background

Many efforts have been made to identify genetic mutations and modulated expression of genes in cytogenetically normal acute myeloid leukemia (CN-AML) that allow further sub-classification and possibly risk-directed therapeutic intervention. Recently, two or three prognostic markers were combined to define patient subgroups with distinct prognosis. But the molecular heterogeneity of CN-AML is not fully reflected in current classification systems.

Purpose

To integrate available clinical and molecular prognostic information for CN-AML patients into one risk score for improved outcome prediction.

Patients and Methods

275 CN-AML patients from multicenter treatment trial AML-SHG Hannover 0199 (ClinicalTrials Identifier: NCT00209833) and AML-SHG Hannover 0295 trial were evaluated for mutations/polymorphisms in NPM1, FLT3, CEBPA, MLL, N-RAS, IDH1, IDH2 and WT1 by direct sequencing. BAALC, ERG, EVI1, MN1, PRAME, and WT1 transcript levels were quantified by RT-PCR. Prognostic markers were integrated into a risk score, which was validated in an independent patient cohort.

Results

The integrative prognostic risk score (IPRS) was modelled in 181 CN-AML patients to represent patients with low, intermediate, and high risk of death. Complete remission rate (CR, P=.005), relapse-free survival (RFS, P<.001), and overall survival (OS, P<.001) were significantly different for the three risk groups. In an independent validation cohort of 94 patients, the risk score predicted different OS (P<.001) and RFS (P<.001). The value of allogeneic stem cell transplantation (SCT) in first CR compared to high-dose cytarabine was evaluated by intent-to-treat analysis in all 225 evaluable patients. Patients in the high-risk group with a related donor had longer OS (P=.016) and RFS (P=.026) compared to patients without a related donor. In contrast, intermediate-risk group patients with a related donor had shorter OS (P=.003) and RFS (P=.05) compared to patients without a related donor. Donor availability had no impact on the outcome of patients in the low-risk group.

Conclusion

We propose a weighted prognostic risk score of clinical and molecular prognostic markers in younger CN-AML patients that may become useful for outcome prediction of currently available consolidation treatment options. This score may be expanded when new markers are discovered, and it may be used to evaluate the efficacy of novel drug treatments in specific subsets of AML patients.

AML

[12] ONCOHIST inhibits survival of human leukemia cells

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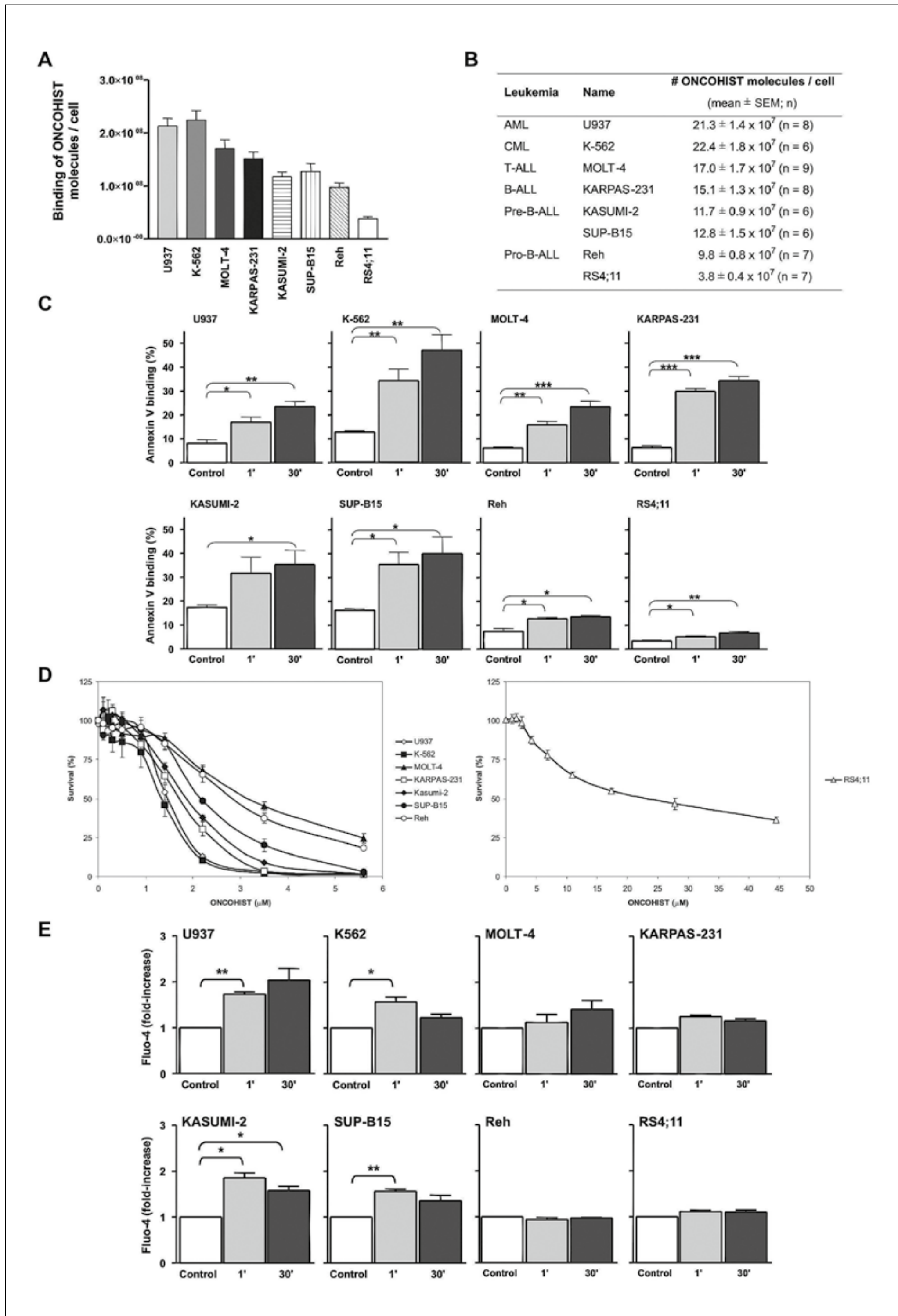
[2] Symbiotec GmbH, Science Park 1, Saarbruecken, Germany.

Objectives and background
Identification of novel drugs that kill cancer cells will provide new therapeutic interventions for leukemia patients. ONCOHIST is a recombinant human histone H1.3 and bis-Met-histone H1.3 drug that derives from histone H1 and was previously tested in patients with acute myeloid leukemia (AML). The safety of ONCOHIST was obtained in the first phase I/II clinical trial with 22 terminally ill AML patients. The clinical trial results were promising; ONCOHIST was tolerated without side effects and first signs of therapeutic effects were observed, such as blast reduction, increase of thrombocytes, and normalization of leukocytes in several patients. To understand ONCOHIST effect on cancer cells we have evaluated binding, apoptosis, and cytotoxicity not only on AML cells, but also on chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cells.

Methods
ONCOHIST molecules that bind to each leukemia cell were quantified by HPLC. Cells were incubated for 30min with 4.4µM of ONCOHIST and protein concentration in the cell supernatant was determined. Apoptosis was evaluated by phosphatidylserine (PS) exposure in the outer membrane leaflet. Cells were incubated with 5.6µM of ONCOHIST for 1 and 30min and stained with annexin-V. Controls are stained cells incubated for 30min in medium without ONCOHIST. Viability assays were performed in the presence of different ONCOHIST concentrations. Cell viability was determined by Alamar blue at 48h. Intracellular Ca^{2+} was determined by staining with Fluo-4. Controls were performed as above. Results are shown as mean ± s.e.m. Statistical significance was determined by Student's t-test (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

Results and statistical significance
We observed that ONCOHIST binds to leukemia cells. We were able to quantify the number of ONCOHIST molecules binding to each cell; varying from $3.8x10^7$–$22.4x10^7$ molecules per cell (Figure 1A, 1B). Incubation of leukemia cells with ONCOHIST for only 1min resulted in a 12.6-35.6% of PS exposure on all cell lines with exception of RS4;11. This exposure slightly increased after 30min. Incubation of RS4;11 did not result in high PS exposure suggesting either a different action mechanism or it might be a reflection of the binding assay results (Figure 1C, 1A). We have also discovered that ONCOHIST has a cytotoxic effect on all leukemia cells tested, and that the IC_{50} varied between 1.3-17.5µM (Figure 1D). Intracellular Ca^{2+} increase was observed in several leukemia cells (Figure 1E) suggesting its involvement in the cytotoxic mechanism of ONCOHIST.

Conclusion
Our observations demonstrate that ONCOHIST binds to leukemia cells and that these cells express functional receptors for ONCOHIST, since binding of ONCOHIST triggers apoptosis and cytotoxicity. Moreover, the mechanism of action of ONCOHIST is quite rapid since only after 1min of exposure leukemia cells became apoptotic. In summary, our results demonstrate that all leukemia cell lines tested are responsive to ONCOHIST, although RS4;11 cells are less sensitive. It is noteworthy that this cell line carries the t(4;11) chromosomal rearrangement that is associated with a greater resistance also to conventional therapies. Overall, ONCOHIST might constitute a promising drug for the treatment of AML, CML and ALL.


A
Binding of ONCOHIST molecules / cell
U937
K-562
MOLT-4
KARPAS-231
KASUMI-2
SUP-B15
Reh
RS4;11
B
Leukemia | Name | # ONCOHIST molecules / cell (mean ± SEM; n)
AML | U937 | 21.3 ± 1.4 x 10^7 (n = 8)
CML | K-562 | 22.4 ± 1.8 x 10^7 (n = 6)
T-ALL | MOLT-4 | 17.0 ± 1.7 x 10^7 (n = 9)
B-ALL | KARPAS-231 | 15.1 ± 1.3 x 10^7 (n = 8)
Pre-B-ALL | KASUMI-2 | 11.7 ± 0.9 x 10^7 (n = 6)
SUP-B15 | 12.8 ± 1.5 x 10^7 (n = 6)
Pro-B-ALL | Reh | 9.8 ± 0.8 x 10^7 (n = 7)
RS4;11 | 3.8 ± 0.4 x 10^7 (n = 7)
C
Annexin V binding (%)
Control
1'
30'
D
Survival (%)
ONCOHIST (μM)
U937
K-562
MOLT-4
KARPAS-231
Kasumi-2
SUP-B15
Reh
RS4;11
E
Fluo-4 (fold-increase)
K562
Control
1'
30'

AML

[13] AML1 gene point mutations in children with acute myeloid leukemia

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Objectives and background
Transcriptional dysregulation is one of the most important leukemia hallmarks. Recently, several critical players involved in this process have being characterised. Among them is the protein encoded by the RUNX1 gene. A number of studies suggest that point mutations in the Runt domain of the RUNX1 gene are also critical for primary and especially treatment- or radiation-induced myelodysplastic syndrome (MDS) and AML formation. Based on this data, we have made a decision to perform the subsequent investigation of the role of AML1/RUNX1 gene mutations in leukemogenesis, particularly in childhood acute myeloid leukemia.

Methods
Patients diagnosed as de novo AML (n=198) and secondary AML (n=9) were included in this study. All of them were Belarusians, aged 0 to 18 years old. Mutational analysis of the AML1 gene exons 3-8 was performed for all patients using SSCP and automated sequencing. For patients with mutations in the AML1 gene, additional analysis of the NRAS gene was carried out to characterise cooperative pathways of leukemogenesis according to a two-hit model.

Results
The frequency of RUNX1 gene point mutations in the total group of patients with de novo AML was 3%. Cooperation of point mutations in RUNX1, NRAS genes and cytogenetic abnormality -7/7q- was demonstrated in children with secondary AML. For patients with childhood de novo acute myeloid leukemia, RUNX1 point mutations predominate in the group with normal karyotypes in leukemic cells. Populational analysis in the group of children with de novo AML from 0 to 14 years-old and diagnosed in the period from 1998 to 2009 has revealed that the frequency of RUNX1 point mutations is about 4%.

Conclusion
In the course of this investigation, valuable data was obtained concerning RUNX1 gene point mutation frequency in different clinical, morphological and cytogenetic groups of patients with myeloid malignancies, and its cooperation with other molecular aberrations. This is the first time, to the best of our knowledge, such a study was done for a certain country population in order to characterise the frequency and appearance of RUNX1 point mutations in childhood AML.

MDS

[14] FISH reveals TET2 deletions in 28 patients with MDS and AML

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Background/Objectives
The present study was aimed at establishing the incidence of band 4q24 deletions/structural defects in 28 MDS/AML examined between January 1993 and January 2009 and to test whether TET2 deletions were correlated with any peculiar gene mutation and clinical findings. Our patients were 12 females and 16 males whose median age was 47 years (range 25-68).

Results
Conventional cytogentic (CC) analysis revealed a normal karyotype in nine patients, a single defect in four, two defects in one and a complex karyotype (with ≥3 abnormalities) in fourteen. Considering the nineteen abnormal patients, eight did not present any defect involving the long arm of chromosome 4, two presented a trisomy 4 (+4), two a monosomy 4 (-4) and all the others harboured structural chromosome 4 defects as either a single abnormality or as a part of a complex karyotype. FISH was carried out with the 144B4 (mapped at 14q22.3), 810D13, 571L19, 414I7 (all mapped at 4q23), 356L5 and 16G16 (both covering the TET2 gene at band 4q24), 642P17, 788K3, 752J12 (all mapped at 4q24) and 66J16 (mapped at 4q25) probes. All these probes were obtained from BACPAC Resources Center at C.H.O.R.I. (Oakland, USA), labelled and applied as previously reported. The cut-off values for interphase FISH (i-FISH) were obtained from the analysis of 300 nuclei from ten normal samples and were fixed at 10%. FISH analysis did not reveal any rearrangement of the TET2 locus in eighteen patients, confirmed +4 in two and -4 in one. In other six patients, FISH identified imbalances involving this chromosomal locus. In particular, in the patient with the t(1;4)(p36;q24) rearrangement, FISH showed the loss of the RP11-356L5, RP11-16G16, RP11-788K3 and RP11-642P17 probes and the maintenance of the RP11-752J12, RP11-66J6, RP11-144B4, RP11-571L19, RP11-810D13 probes in 88% of interphase and mitotic cells examined. So, this patient presented a loss of the TET2 gene and of the RP11-788K3, RP11-642P17 probes, even if the chromosomal translocation breakpoint was localized at band 4q25. In another patient with an insertion of chromosome 4 material from band q24 to band qter into 5q on CC, FISH showed a cryptic deletion of the chromosomal segment between bands 4q22.3 (RP11-144B4) and 4q24-q25 (RP11-752J12) and a translocation of the RP11-66J6 probe, which resides at 4q25, into der(5). In two patients, FISH revealed a cytogenetic cryptic deletion which in the first case involved all the probes tested, whereas in the second one involved the RP11-571L19, RP11-365I5, RP11-16G16, RP11-642P17, RP11-788K3 and RP11-752J12. The last patient with additional material on band 4q33 presented a deletion of probes RP11-144B4, RP11-365L5, RP11-16G16, RP11-642P17, RP11-752J12 and RP11-66J6. Interestingly, probes RP11-810D13, RP11-571L19 and RP11-788K3 were maintained.

Conclusions
In conclusion, i) FISH is a good method for identifying cryptic TET2 deletions, as the chromosomal area containing this gene is often deleted independently of the chromosomal breakpoints; ii) TET2 deletions are rare events occurring at an incidence of 5.1%. The 17.8% frequency of our study is probably due to bias in sample collection; iii) TET2 deletion is not always associated with chromosome 4 rearrangements on conventional cytogenetics.

MDS

[15] Unexpected karyotype lesions are revealed by FISH with probes derived from array CGH (aCGH) in chromosomally normal MDS patients

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In MDS, the cytogenetic pattern is one of the most important parameters to predict overall survival (OS) and the risk of MDS/AML evolution. However, in about 40-50% of patients, especially low- risk MDS, conventional cytogenetics (CC) fails to reveal clonal chromosomal defects. FISH with probes specific for chromosomal regions most commonly involved in MDS can improve CC results (Bernasconi et al., 2003). Currently, aCGH revealed that MDS patients, independent of the cytogenetic pattern, harbour novel chromosomal lesions targeting unsuspected regions.
Thus, our study was aimed at evaluating whether probes derived from a recent aCGH were truly able to unmask cryptic lesions in chromosomally normal MDS patients and whether they influenced OS and disease evolution. The thirty-four patients entered the study were examined between January 2005 and May 2010. They were twelve females and twenty-two males; their median age was 64 years (range 24-75). According to WHO classification, 6 patients were classified as RA, 4 as RCMD, 11 as RAEB-1 and 13 as RAEB-2. Considering IPSS score, 9 patients were considered low-risk, 13 intermediate-1 risk and 12 intermediate-2 risk. Median follow-up was twelve months (range 1-46). At the time of the study no patients have died, whereas 7 have evolved to RAEB-2 and 4 to AML. FISH probes were chosen based on the frequency of their involvement in MDS and their Mb position determined using UCSC genome browser on Human Mar. 2003 assembly. They were obtained from BACPAC Resources Center at C.H.O.R.I. (Oakland, USA), labelled and applied as previously described. We used the following probes: RP11-912d8 (19q13.2); RP11-196p12 (17q11.2); RP11-269c4 (14q12); RP11-351o1 (10q21.3); RP11-144g6 (10q11.2); RP11-122a11 (7q34); RP11-951k18 (5q13.1); RP11-100m20 (4p14); RP11-544h14 (2q33). An abnormal FISH pattern was revealed in 16 patients (47.0%): 7 presented a 19q13.2 deletion, 3 an amplification of band 4p14, 2 a 14q12 deletion, 2 a deletion of 7q34, one a deletion of band 17q11.2, and one a potential rearrangement of band 10q11.2. One of these patients harboured two defects, namely a 19q13.2 deletion and an amplification of band 4p14. An abnormal FISH pattern was observed in 2/6 RA patients, in 2/4 RCMD, in 6/11 RAEB-1 and in 6/13 RAEB-2 and in 3/9 IPSS low-risk, in 7/13 intermediate-1 risk and in 6/12 intermediate-2 risk MDS patients. Disease evolution occurred in the only 2 RA patients with an abnormal FISH pattern and in 6 of the 24 RAEB-1/RAEB-2 patients with an abnormal FISH pattern. Additional studies are warranted to assess the prognostic significance of cryptic chromosomal lesions.

In conclusion, our data suggest that FISH with probes derived from aCGH studies: i) reveals novel unsuspected chromosomal lesions in about 47% of chromosomally normal MDS patients; ii) these chromosomal lesions mostly consist in gains/losses, whereas balanced rearrangements seem to be very rare; iii) an abnormal FISH pattern seems to correlate with disease progression even if this observation should be confirmed on additional patients.

[16] Gene expression profiling of pediatric MDS characterises time to progression into AML

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Objectives and background
Myelodysplastic syndrome (MDS) is a heterogeneous group of stem cell disorders characterised by ineffective hematopoiesis and a varying propensity to evolve into acute myeloid leukemia (AML). In adult MDS, several studies based on gene expression profiling (GEP), mostly using purified CD34+ or AC133+ cells, have been reported. So far only few studies of GEP analysed MDS pediatric patients, which may be due to the rarity of this disease. We report a GEP analysis of pediatric MDS patients to contribute to the diagnosis of this disorder and to understand molecular pathways involved in the progression of pediatric MDS into acute myeloid leukemia (AML).

Methods
We analysed 24 samples at diagnosis of MDS. Patients were divided according to pediatric WHO classification: 12 out of 24 (50%) were diagnosed as refractory cytopenia (RC), 7 samples (29%) as refractory anemia with excess of blasts (RAEB) and 5 (21%) as RAEB in transformation (RAEB-t).
Moreover, we analysed 17 AML with normal karyotype and 8 normal bone marrow samples (pediatric age).
Patients' parents or their legal guardians provided written informed consent following the tenets of the Declaration of Helsinki.
We performed gene expression analysis of a total of 49 pediatric patients using the HG U133 Plus 2.0 array. For statistical analysis we used Partek Genomic Suite Software and R package.

Results
The unsupervised analysis of the total of 49 samples divided most of the MDS samples from AML and normal bone marrow. In details, only 6 patients at diagnosis of MDS clustered together into the branch of AML. Interestingly, 4 of these patients evolved in AML within 1 year from diagnosis, whereas 3 MDS patients that transformed into AML after more than 1 year cluster in the MDS branch. Based on the results of the unsupervised clustering analysis, we performed a supervised analysis comparing the MDS specimens that evolve in AML within versus after 1 year from diagnosis. We found 214 differently expressed probe sets (FDR < 0,05) between these two groups.
Remarkably, MDS patients that evolved in AML within 1 year showed up-regulation of several genes known to be involved in AML i.e. HOX cluster genes, WT1. The latter genes have prognostic significance particularly in AML patients with normal karyotype.
On the contrary, among the up-regulated genes in MDS samples that evolved in AML later than 1 year, we identified genes such as KLF4, DPPA4 that are known to be involved in cellular stemness.

Conclusions
We reported a gene expression study focused on the molecular pathways that are involved in the progression of pediatric MDS into AML. We identified two signatures characterising patients that evolved in AML within or after 1 year from diagnosis. Thus, patients that evolved in AML within 1 year showed already at diagnosis the up-regulation of genes involved in AML development. Interestingly, these patients also showed the down-regulation of KLF4, a potential tumour suppressor that has been reported to be involved in solid tumours progression.

MDS

[17] 5'-Azacytidine in higher-risk MDS and AML: A single center experience

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Background
Treatment options in patients (pts) with higher-risk (HR) MDS and secondary or primary refractory AML are limited. 5'-azacytidine (AZA) prolongs survival in HR MDS pts in comparison with supportive care including hydroxyurea and low dose AraC (LD-AraC) (Fenaux et al, Lancet Oncol 2009). However, data outside clinical trials and in AML remain scarce.

Patients and Methods
Patients (pts) treated in our clinic with AZA 75 mg/m^2 SC 7 days/4 weeks from drug approval in Greece, were included.

Results
34 pts with HR MDS (IPSS int-2/high) and secondary or primary refractory AML were treated with AZA between 1/6/09 and 15/7/10. Median age was 70 (30-77), M/F ratio was 3 and median time from diagnosis was 35 months (0.25-114). WHO classification was: 6% RA, 12% RAEB-1, 12% RAEB-2, 11% unclassifiable MDS/MPN, 44% secondary AML (26% RAEB-T), 15% refractory de novo AML (6% primary refractory and 9% refractory relapses). 10% of pts had treatment-related MDS/AML. Karyotype was favourable, intermediate, unfavourable according to IPSS in 37%, 50% and 13% of pts, respectively. Median leukocyte count and % of blasts in bone marrow (BM) and peripheral blood (PB) were 5.9 G/L, 20% and 5%, respectively. Median number of AZA cycles was 3 (1-9) with a median interval of 28 days. AZA had been administered after failure of intensive chemotherapy, AraC bolus and LD AraC in 30%, 18% and 8% of pts, respectively. Dose reduction and hydroxyurea addition were necessary in 12% and 21% of pts, respectively. Response rate (RR), according to IWG 2006 criteria in 28 evaluable pts, was 39% (CR 3%, PR 7%, marrow response 11%, hematological improvement (HI) 18%), with 23.5% of pts having stable disease. Median response duration was 9 months (3-9). Age, sex, leukocyte count, % of BM blasts, WHO classification, primary or secondary character, karyotype and previous chemotherapy did not influence RR while the % of PB blasts and the number of AZA cycles were associated with higher RR (57% vs. 21% in pts with ≤4% and >4% PB, P=0.049, and 60% vs. 15% in pts with >3 and ≤3 cycles, resp., P=0.013). 2/2 pts with MDS/MPN and JAK2 V617F responded (HI). Two pts underwent allogeneic SCT following AZA (1 non responder and 1 in PR after 1 and 6 cycles, resp.). Median number of days spent in hospitalization was 0 (0-48). 3 pts experienced serious events attributable to AZA: Lactic acidosis with encephalopathy, ischemic colitis and fatal cerebral hemorrhage. Bacteremia and/or pneumonia during treatment occured in 2 pts. With a median follow-up of 5 months, median overall survival (OS) from treatment onset was 11.3 months. OS was increased in pts who received >3 cycles of AZA (median OS 11.3 vs. 3 months in pts with ≤3 cycles, P=0.016) and tended to be higher in responders vs. non responders (median 11.3 vs. 8 months, resp., P=0.08).

Conclusions
AZA was well tolerated in pts with advanced MDS/AML and permitted management in an outpatient basis. Response rates may be higher when treatment is administered for > 3 cycles.

MDS

[18] Karyotype evolution and rare abnormalities in MDS patients detected from peripheral blood by sequential FISH analyses on circulating CD34+ cells: Results of the prospective multicenter German diagnostic study

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Objectives and background

In myelodysplastic syndromes (MDS), chromosomal anomalies are important for pathogenesis, prognosis, diagnostics and treatment allocations. Usually, they are detected by conventional chromosome banding analyses of bone marrow (BM) cells, but most of them are provable by fluorescence in situ hybridisation (FISH) of circulating CD34+ cells from peripheral blood (PB), as well. To detect chromosomal aberrations from PB, to follow the clone sizes by close-meshed sequential FISH analyses and to correlate the molecular-cytogenetic results with BM morphology and clinical data, we started a prospective multicenter German diagnostic study in 10/2008.

Methods

Sequential FISH analyses were performed on immunomagnetically enriched circulating CD34+ cells from PB using a "superpanel" (D7/CEP7, EGR1, CEP8, CEP XY, D20, p53, IGH/BCL2, TEL/AML1, RB1, MLL, 1p36/1q25, CSF1R) for initial screening, every 12 months during follow-up and in every case of suspected progression and a "standard panel" (EGR1, D7/CEP7, CEP8, p53, D20, CEP X/Y, TEL/AML1) for close-meshed analyses every 2 months in the 1st year and every 3 months in the 2nd year. Every 6 months a BM biopsy is suggested including cytomorphology, chromosome banding and FISH analyses from BM blood for correlation with the results from PB. These data together with PB counts once a month and treatment modalities were documented in a database.

Results

As yet, 205 pts were included in the study. The study cohort was representative for MDS in sex, age and MDS subtypes (according to WHO and IPSS). In 62% of pts (126/205), chromosomal aberrations were detectable by FISH from PB. The most common aberrations were a 5q-deletion (64%), aberrations of chromosome 7 (del(7q)/-7) (25%), an allelic loss of p53 (12%), +8 (11%), 20q-deletion (10%), loss of the Y-chromosome (7%) and aberrations of chromosome 21 (del(21q)/-21/+21) (7%). Interestingly, a 12p-deletion was detectable in 11% of pts with aberrant karyotypes, which is more often than it was described in literature before. In most cases the del(12p) was part of complex aberrations. In 4 pts, there was a coexistence of del(7q) and -7 at the same time. And 10/15 pts (67%) with an allelic loss of p53 showed an aberration of chromosome 7 at the same time.

A karyotype evolution could be diagnosed from PB in 17 pts (13%) out of 135 with at least 2 analyses within a median observation time of only 7 months (2-21). The most frequent secondary anomaly was del(7q)/-7, followed in frequency by aberrations of chromosome 21.

Conclusions

We could demonstrate that FISH analyses of circulating CD34+ cells are a feasible, reliable and less invasive method to detect chromosomal aberrations in MDS pts and to follow the clone sizes in PB. By close-meshed sequential analyses we were able to observe karyotype evolution in detail, to detect new aberrations during follow-up and under therapy, and to follow even rare abnormalities to learn more about their prognostic impact.

MDS

[19] Comparison of 5-azacytidine versus allogeneic hematopoietic cell transplantation in elderly (≥ 60 years) patients with de novo high-risk MDS - a retrospective matched pair analysis

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Background

Myelodysplastic syndromes (MDS) are a disease of the elderly in whom until recently, allogeneic hematopoietic cell transplantation (HCT) has not been considered a reasonable therapeutic option. Best supportive care (BSC), only including transfusion support, has been the standard of care for the majority of these patients (pts). However, innovations in transplant protocols have changed that approach, resulting in an increased frequency of allogeneic HCT. Further, several innovative non-transplant therapeutic modalities for MDS pts have been developed. In fact, treatment with DNA-methyltransferase inhibitors (MTI) such as 5-azacytidine (5-aza) has changed the natural course of the disease and prolonged median survival by an average of 9 months compared to BSC. However, allogeneic HCT is currently still the only modality with proven curative potential, although there has been no true randomized trial comparison of HCT to 5-aza therapy only.

Methods

Consequently, we performed a retrospective analysis in 126 pts, 60 -77, (median 64) years of age with de novo high-risk MDS according to FAB classification (RAEB n=88, RAEB-t n=20, CMML n=18) undergoing allogeneic HCT at a median of 9 months from initial MDS diagnosis. Cytogenetics were available in 108 pts (good: n=63, intermediate: n=18, poor: n=27), resulting in IPSS classification (in 107 pts) of INT-1 (n=28), INT-2 (n=45) or HIGH (n=34). The ECOG was available in 125 pts, and was either 0 (19%), 1 (66%) or >1 (15%). Prior to HCT, most pts had progressed to a higher stage than present at diagnosis (RAEB n=45, RAEB-t n=10, CMML n=10, AML n=61), the median blast count in the marrow being 12%. Patients were prepared for HCT with one of several reduced (RIC, n=78) or more conventional intensity (n=48) conditioning regimens followed by stem cells from either related (n=50) or unrelated (n=76) donors.

The outcome of this high-risk MDS cohort was compared to patients from the French (GFM) 5-aza patient registry matched for age, gender, prior induction chemotherapy, time from diagnosis to therapy (either HCT or 5-aza), disease stage and cytogenetics at diagnosis as well as at start of therapy. The groups could be well matched except for age (median age 5-aza: 67 vs. HCT: 64; p=0.001). Further, the HCT group included more pts with a higher ECOG score (ECOG>0: 81% vs. 59%; p=0.05) prior to start of therapy.

Results

With a median follow-up of 20 months for surviving pts from the start of therapy, the estimated 3-year overall survival (OS) was 39% (95% CI, 30% to 48%) for HCT and 7% (95% CI, 0 to 16%) for 5-aza, respectively. In multivariate Cox regression analysis, ECOG score (p<0.001), disease stage p=0.021), cytogenetic risk group (p=0.002) and type of treatment (5-aza vs. HCT HR: 1.8; p=0.003) were associated with survival.

Conclusion

These retrospective data suggest that allogeneic HCT from related or unrelated donors, using various conditioning approaches, might offer a meaningful survival benefit compared to 5-aza in patients with high-risk MDS, even in the 7th decade of life. There is a need for prospective clinical trials in order to determine the place of this approach within the growing therapeutic opportunities for pts with MDS.

MDS

[20] NQO1 (C609T) polymorphism and specific types of chromosome aberrations in myelodysplastic syndromes (MDS)

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Objectives and background
MDS are characterised by acquired clonal chromosome aberrations closely associated with the leukemogenic process. Models for the MDS pathogenesis imply the role of cumulative genetic and xenobiotic factors in genetically predisposed individuals. The widely expressed NAD(P)H:quinone oxidoreductase 1 (NQO1) enzyme is involved in the cellular response to oxidative damage protecting cells from quinones, the ultimate toxic metabolites after exposure to benzene. The encoding gene is subject to the genetic single nucleotide polymorphism C609T, changing the amino acid sequence (Pro187Ser), resulting in enzymatic inactivation. Individuals homozygous for the mutant allele (T/T) completely lack NQO1 activity, whereas heterozygotes (C/T) present threefold decreased enzymatic activity.
We hypothesised that the NQO1 gene polymorphism may predispose individuals to a greater risk of MDS and/or promote specific types of chromosome aberrations. To investigate the potential role of NQO1 C609T inborn polymorphism in MDS pathogenesis, we performed a case-control study analyzing the NQO1 genotypic distribution in a large group of Greek patients with primary MDS. We next compared the genotypic frequencies in patients with isolated +8, del(5q) and -7/del(7q), since these represent the commonest aberrations in MDS and may indicate prior exposure to xenobiotics.

Methods
The NQO1 C609T polymorphism was investigated in 261 MDS patients and 270 matched healthy controls using a PCR-RFLP assay. It was evaluated in respect to patient characteristics, chromosome abnormalities and IPSS classification. The NQO1 genotype was additionally investigated in 80 MDS patients carrying the most common unbalanced abnormalities +8, del(5q) and -7/del(7q) as sole abnormalities.

Results and statistical significance
A cytogenetic result was achieved in 255/261 patients at diagnosis. Among them, 103 (40.4%) showed clonal karyotypic abnormalities. The most frequently observed single abnormality was +8 (25/255, 9.8%). The distribution of the NQO1 genotypes did not differ between MDS patients and controls: homozygous wild type (C/C) 65.1 vs 61.2%; heterozygotes (C/T) 33 vs 37%; homozygous mutant (T/T) 1.9 vs 1.8%. Stratification of patients according to gender, age and IPSS groups revealed no differences in the mutant (C/T and T/T) genotypic frequencies.
To investigate the impact of NQO1 polymorphism in the pathogenesis of certain recurrent karyotypic imbalances, we additionally analyzed 80 MDS patients with +8 (n=44), del(5q) (n=29) and -7/del(7q) (n=7) as sole abnormalities. We observed no differences in the frequencies of NQO1 polymorphism between patients with del(5q) and healthy controls. On the contrary, the NQO1 polymorphism was associated to an increased risk of MDS with isolated chromosome 7 abnormalities ($p<0.05$). Moreover, an increased frequency of the homozygous mutant genotype (T/T) was observed in patients with isolated trisomy 8, as compared to the controls (9.1% vs 1.8%, $p<0.05$).

Conclusions
Our results show that the NQO1 C609T polymorphism does not correlate with susceptibility to MDS. The high incidence of NQO1 mutant genotype in patients with -7/del(7q) is noteworthy, since chromosome 7 aberrations are indicators of previous exposure to benzene. The high frequency of the homozygous mutant genotype in our patients with isolated trisomy 8, reflecting lack of NQO1 enzyme activity, might confer a subgroup with defined prognostic significance within the heterogeneous population of +8 patients.

Other myeloproliferative neoplasms

[21] Epigenetic dysregulation of secreted Frizzled-related proteins in myeloproliferative neoplasms

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Introduction
Hypermethylation of CpG islands within gene promoter regions is associated with transcriptional inactivation and represents an important mechanism of gene silencing in the pathogenesis of hematopoietic malignancies. Myeloproliferative neoplasms (MPN) constitute a group of hematopoietic malignancies and comprise chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). BCR/ABL-negative MPN (Ph-MPN) are typically characterized by an autoactivating mutation in the JAK2 tyrosine kinase (JAK2V617F) as well as additional mutations in the JAK-STAT pathway. Secreted Frizzled-related proteins (SFRPs) are antagonists of the Wnt signaling pathway. Consistent with the central role of the Wnt pathway for stem cell maintenance and differentiation of hematopoietic progenitors, epigenetic downregulation of SFRPs has been shown in hematopoietic malignancies.

Methods
We determined the promoter methylation status of SFRP1, -2, -4 and -5 in human cell lines and in 60 samples from MPN patients by methylation-specific PCR (MSP). The JAK2V617F mutation was assessed by allele-specific PCR.

Results
Aberrant methylation among primary MPN samples was 5 % (3/60) for SFRP-1, 24 % (14/60) for SFRP-2, 2% (1/60) for SFRP-4 and 0% (0/60) for SFRP-5. The SFRP-2 promoter was predominantly methylated with 0% in CML (0/12), 27% in PV (3/11), 30% in ET (3/10) and 26% in PMF (7/27). There was no correlation between SFRP-2 hypermethylation and MPN subtype. We detected a trend for a correlation between methylation of the SFRP-2 promoter and presence of JAK2V617F mutation (p = 0,1).

Discussion
Our data indicate that epigenetic dysregulation of the Wnt signaling pathway is a common event in Ph-MPN with aberrant methylation of at least one SFRP being detected in 30% of the primary patient samples with the most predominant member of the family, SFRP2 being methylated in about one-third of the cases. Taken together, aberrant methylation of crucial stem cell maintenance genes seems to contribute significantly to disease pathogenesis in Ph-MPN.

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