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ABSTRACTS

ELN Frontiers Meeting 2012

MYELOID NEOPLASMS: APPROACHING CURE

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9-11 November 2012, Swissôtel The Bosphorus, Istanbul, Turkey
Dear colleague,

we are delighted to welcome you to the ELN Frontiers Meeting 2012 entitled ‘Myeloid Neoplasms: Approaching Cure’. This official meeting of the European LeukemiaNet (ELN) is taking place on November 9-11 at the Swissôtel The Bosphorus in Istanbul, Turkey. The meeting is co-sponsored by the University of Heidelberg and the Turkish Society of Hematology, and it is endorsed by EUTOS for CML.

The ELN Frontiers meeting is dedicated to provide an opportunity to facilitate discussions with renowned experts in hematology from all across Europe and to share new developments in the field of myeloid neoplasms. Recognising important recent advances in the diagnosis and targeted treatment, ELN Frontiers 2012 will focus on chronic myeloid leukemia (CML), myeloproliferative neoplasms (MPN), myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). The curriculum will discuss major treatment advances, molecular and diagnostic tools, clinical management issues and promising clinical research in these disease areas.

In the past two decades, we have witnessed dramatic advances in the management of myeloid neoplasms. Second generation tyrosine kinase inhibitors are now proven to achieve fast, deep and sustained responses in first-line treatment of CML patients. Thus, clinical response milestones in CML are expected to evolve once again. The recent availability of Janus kinase inhibitors for the treatment of MPNs has already led to significant quality of life improvements for patients. New breakthroughs in our understanding of the molecular pathogenesis of MDS and AML will impact the future management of these diseases.

Please join us on the poster walks which recognise and disseminate the contributions of researchers in this field. Accepted poster abstracts may be found in our special edition of the ELN Newsletter and many will be presented orally. We encourage you to exchange discussion on your research.

The ELN Frontiers meeting builds upon 6 years of education innovation in myeloid malignancies. Please actively participate in this exciting congress with a rich mix of plenary and interactive parallel sessions including meet-the-experts, clinical case discussions, debates, poster walks and oral presentations.

Yours sincerely

M. Baccarani and R. Hehlmann
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Background
Despite the consistent nature of the BCR-ABL1 fusion gene identified at diagnosis, CML is clinically heterogeneous as exemplified by the predictive value of Sokal scores and the variable response to tyrosine kinase inhibitors (TKI). We speculated that aberrant epigenetic programming and in particular differential DNA methylation might underlie the variations in outcome and variable responses to imatinib.

Methods
CD34+ cells were purified from the blood of 46 patients with CML-CP at diagnosis. Patients were treated with imatinib (400mg/day) and classified as responders (n=29) if they achieved durable complete cytogenetic response (CCyR). Responders were subjected to leukapheresis following G-CSF mobilization. Non-responders (n=17) never attained major cytogenetic response (MCRy) or lost a previously attained CCyR despite continuing imatinib (n=4). Using the Illumina Infinium HumanMethylation450 BeadChip to scrutinize 429,231 CpG dinucleotides within the genome, the pre-treatment genome-wide methylation profiles of the responders were compared with those of the non-responders as well as with the corresponding paired CCyR sample. Analogous methylation signatures were obtained from CD34+ cells collected from healthy donors (in excess of requirement) treated with G-CSF to yield cells destined for allogeneic stem cell transplantation. Using Random Forest (RF), an ensemble classifier (super-learned algorithm) together with a repeated sampling method of the important features selected, it was possible to select a subset of probes for use in a classification model to separate imatinib responders from non-responders based on their genome-wide DNA methylation profile.

Results
Unsupervised hierarchical clustering of all samples using Ward’s method showed that genome-wide DNA methylation analysis clearly distinguishes between CML-CP, CML-CCyR and normal CD34+ cells. Two clusters, one derived from diagnostic CML samples and the other from the controls and CCyR samples (i.e. minimal or no leukemia) were identified. In the latter cluster CCyR samples were distinct but epigenetically much closer to normal CD34+ cells than CML-CP samples. 109 different probes separated responders from non-responders and displayed increased co-methylation compared to a background set. 81% of these probes contained within CGI demonstrated higher methylation values in non-responders than responders. Using these 109 probes a ‘leave-one-out’ cross-validation procedure was repeated 100 times to estimate their prediction accuracy; their use correctly predicted all responders.

Conclusions
The work so far has identified consistent differences in genome-wide methylation patterns between patients with untreated disease versus normal controls indicating a possible common epigenetic pathway in the leukemic transformation process. The study has also defined a 109-probe DNA methylation classifier, which predicts overall response to imatinib in CML patients. Validation with a second independent patient cohort is currently in progress.

[1] A DNA Methylation Classifier Predicts Patient Response to Imatinib in Pre-Treatment CML Samples
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Objective and background
Definitive spectra of point mutations in the BCR-ABL kinase domain (KD) confer resistance to tyrosine kinase inhibitors (TKIs) in Philadelphia-positive (Ph+) leukemias. The presence/absence of mutations and the type of mutation are two important pieces of information to be integrated in the clinical decision algorithm guiding clinicians in therapeutic decision making. BCR-ABL KD mutations are currently detected using Sanger sequencing (SS) which has a sensitivity of 20%. With the novel ultra-deep sequencing (UDS) technologies, each amplification reaction is sequenced individually thousands of times, thus allowing conjugate results and a higher sensitivity of screening for known and unknown mutations, a high throughput and the possibility to fully characterize the spectrum of minor mutated variants. Additionally, UDS allows accurate quantification of mutant sub clones so as to more carefully follow their dynamics over time. We used an UDS approach in order to:
- Resolve qualitatively and quantitatively the complexity of mutated populations surviving TKIs,
- Investigate their clonal structure and study the dynamics of expansion of mutated clones in Ph+ patients receiving TKI-based therapies,
- Test the ability of UDS to highlight emerging clones harbouring critical mutations.

Methods
We set up a BCR-ABL KD mutation screening strategy on the Roche GS Junior instrument. We designed 4 partially overlapping UDS amplicons covering the KD of the BCR-ABL transcript to be generated by nested RT-PCR using sequence-specific primers conjugated with multiplex identifiers – allowing us to pool and sequence different samples from one or multiple patients (pt s) in a single run. We used this strategy to retrospectively perform a longitudinal analysis of a total of 111 samples from 35 CML or Ph+ ALL patients who had received sequential treatment with multiple TKIs (two to four TKIs among imatinib, dasatinib, nilotinib, ponatinib) and had experienced sequential relapses accompanied by selection of TKI-resistant mutations, as assessed by SS.

Results and significance
UDS proved valuable to identify and quantify sequence variations in samples from patients already known to harbour mutations as assessed by SS with 100% concordance. UDS results showed good reproducibility: five samples sequenced twice in independent runs showed very good concordance in variant detection, even for low level variants, and two samples sequenced twice in independent runs and at different depth showed that variant abundance was similar despite different sequencing depth. Inter-laboratory reproducibility is being assessed in collaboration with an international consortium of 10 laboratories from 8 countries, within the framework IRON Study II (Inter-laboratory Robustness of Next Generation Sequencing).
A preconfigured 96-well plate containing lyophilized primer pairs targeting the ABL KD has been designed and a protocol is being optimized for future diagnostic application. Analysis of patients who developed resistance to multiple TKIs showed that SS may misclassify BCR-ABL KD mutation status or underestimate its complexity in more than
[3] Predictive Value of BCR-ABL Transcript Levels at 3 and 6 Months in Chronic Myeloid Leukemia Patients Treated Frontline with Imatinib Mesylate

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Objectives and Background
The objective was to investigate the prognostic impact of BCR-ABL1 levels at 3 and 6 months on the long-term outcome of CML patients treated frontline with imatinib mesylate (IM) in Italy. IM is the thera- peutic standard for chronic myeloid leuke- mia (CML), but nilotinib and dasatinib have the potential to replace it. The early predic- tion of poor outcome is important to opti- mize the treatment strategy. In IM-treated patients, BCR-ABL transcript levels according to the International Scale (IS) >10% at 3 and >1% at 6 months were able to iden- tify high-risk groups (Marin et al., J Clin On- col 2011; Hanfstein et al., Leukemia 2012). Similar analyses were performed within the IM arms of the ENEStnd trial (Hochhaus et al., EHA 2012) and the DASISION trial (Jabb- bour et al., EHA 2012).

Methods
We analysed 559 patients enrolled within 3 trials of the GIMEMA CML WP (Clini- alsGov NCT00514488/NCT00510926, ob- servational trial CML023). The numbers of patients with evaluable QPCR samples at 3 and 6 months were 487/559 (87%) and 492/559 (88%), respectively. Definitions: major molecular response (MMR): BCR-ABL1 ratio <0.1%; MRA: BCR-ABL1 <0.01%; failure: according to 2009 ELN recommen- dations. The rate of complete cytogenet- ic response (CCR) and MMR and MR4.0 at 1 year, the rate of MR4.0 at 2 years, the failure-free survival (FFS), the progression-free survi- val (FFS) and the overall survival (OS) accord- ing to the BCR-ABL transcript levels (<10% vs >10% and ≤1% vs >1%) at 3 and 6 months were analysed.

Results and Significance
Median age: 52 years (range 18-84). IM dose: 76% 400 mg, 24% 800 mg. Sokal score: 39% low, 39% intermediate, 22% high; EUTOS score: 93% low, 7% high. Median follow-up: 76 months (range: 7-99); 95% of patients had at least 5-year obser- vation. BCR-ABL1 at 3 months: ≤1% in 336/487 (69%), >1% to ≤10% in 120/487 (25%) and >10% in 31/487 (6%). BCR-ABL1 at 6 months: ≤1% in 425/492 (86%), >1% to ≤10% in 54/492 (11%) and >10% in 13/492 (3%). Responses and outcomes according to transcript levels are presented in the attached table. Patients with BCR-ABL1>10% at 3 months achieved inferior CCR and MMR rates at 1 year and inferior MR4.0 rate at 2 years, but the long term out- come was comparable to patients with transcript levels ≤10%. On the contrary, a BCR-ABL1<1% at 3 months was associated, not only with lower subsequent response rates, but also with significantly inferior FFS, PFS and OS. Results were similar, with small differenc- es, in the 6 month analysis.

Conclusions
In a multicentric nationwide experience, the proportion of patients with BCR-ABL1 transcript levels >10% at 3 and 6 months was low. The risk distribution and the proportion of patients treated with high-dose IM may explain, at least in part, the differences with oth- er published reports. At 3 and 6 months, a BCR-ABL1 cut off of 1% was a reliable surrogate marker of response and outcome. A BCR-ABL1 level >1% at 3 and 6 months represents a warning, requiring a close monitoring. A switch to 2nd generation tyrosine kinase inhi- bitors should be considered.

<table>
<thead>
<tr>
<th>BCR-ABL at 3 months (N = 487)</th>
<th>≤ 10% (N = 456)</th>
<th>&gt; 10% (N = 31)</th>
<th>p*</th>
<th>≤ 1% (N = 335)</th>
<th>&gt; 1% (N = 131)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR at 1 year, %</td>
<td>80.9</td>
<td>51.6</td>
<td>&lt;0.001</td>
<td>87.5</td>
<td>69.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMR at 1 year, %</td>
<td>62.1</td>
<td>22.6</td>
<td>&lt;0.001</td>
<td>70.5</td>
<td>51.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MR4.0 at 2 years, %</td>
<td>20.0</td>
<td>3.2</td>
<td>0.017</td>
<td>23.5</td>
<td>8.6</td>
<td>0.006</td>
</tr>
<tr>
<td>FFS, %</td>
<td>79.2</td>
<td>68.3</td>
<td>0.104</td>
<td>93.7</td>
<td>68.6</td>
<td>0.004</td>
</tr>
<tr>
<td>PFS, %</td>
<td>85.2</td>
<td>90.0</td>
<td>0.771</td>
<td>87.5</td>
<td>81.9</td>
<td>0.004</td>
</tr>
<tr>
<td>OS, %</td>
<td>86.5</td>
<td>87.1</td>
<td>0.622</td>
<td>88.4</td>
<td>83.6</td>
<td>0.010</td>
</tr>
<tr>
<td>BCR-ABL at 6 months (N = 492)</td>
<td>≤ 10% (N = 479)</td>
<td>&gt; 10% (N = 13)</td>
<td>p*</td>
<td>≤ 1% (N = 425)</td>
<td>&gt; 1% (N = 67)</td>
<td>p*</td>
</tr>
<tr>
<td>CCR at 1 year, %</td>
<td>84.3</td>
<td>30.8</td>
<td>&lt;0.001</td>
<td>88.7</td>
<td>46.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MMR at 1 year, %</td>
<td>64.7</td>
<td>7.7</td>
<td>&lt;0.001</td>
<td>71.1</td>
<td>13.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MR4.0 at 2 years, %</td>
<td>19.6</td>
<td>0</td>
<td>0.143</td>
<td>21.4</td>
<td>4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FFS, %</td>
<td>81.0</td>
<td>57.1</td>
<td>0.045</td>
<td>83.6</td>
<td>57.4</td>
<td>0.004</td>
</tr>
<tr>
<td>PFS, %</td>
<td>86.4</td>
<td>74.1</td>
<td>0.115</td>
<td>78.6</td>
<td>78.4</td>
<td>0.002</td>
</tr>
<tr>
<td>OS, %</td>
<td>87.3</td>
<td>76.9</td>
<td>0.078</td>
<td>88.3</td>
<td>80.8</td>
<td>0.010</td>
</tr>
</tbody>
</table>

*Responses at each time point were compared using y2 test or Fisher exact test, as appropriate. FFS, PFS, OS were estimated using the Kaplan-Meier method and compared by log-rank test. Patients with events or censored within 3 or 6 months were excluded from the respective analysis.

half of the cases, since multiple lower-level (<20%) mutations were found both in sam- ples that had been scored as wild-type by SS and in samples already harbouring muta- tions with >20% abundance.

Interestingly, in 14/25 (56%) Ph+ ALL pa- tients with molecularly detectable disease but not yet evidence of cytogenetic or hematologic relapse, UDS could identi- fy emerging TKI-resistant mutations 1 to 2 months before they became detectable by SS. In the remaining 11 patients, dynamics of outgrowth of the TKI-resistant mutations (five T315I, two Y253H, two E255V, one E255K and one F317L) was so rapid that not even strict monthly monitoring allowed to be detected before they became dominant.

Conclusions
UDS allows more sensitive and accurate characterization of resistant sub clones in CML and Ph+ ALL patients receiving TKI- based therapies. UDS could soon replace standard methods for diagnostic BCR-ABL KD mutation screening.

(Supported by European LeukemiaNet, PRIN, Fondazione Carisbo, IGA MZCR NT11555.)
[4] A High EUTOS Score Is Predictive For Adverse Outcome in Chronic Myeloid Leukemia Patients Treated Frontline with Nilotinib-Based Regimens: A GIMEMA CML WP analysis

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Objectives and Background

The aim of the present analysis was to investigate the prognostic value of the EUTOS score in a cohort of early chronic phase (CP) chronic myeloid leukemia (CML) patients treated with nilotinib (NIL)-based regimens. The outcome of CP CML has been significantly improved by the introduction of tyrosine kinase inhibitors (TKIs); NIL is a 2nd generation TKI with superior efficacy to imatinib (IM), and approved as a front-line therapy. Until recently, the prognosis of CML patients has been evaluated using prognostic scores developed in the chemotherapy and interferon era. The EUTOS score, a new scoring system based on the analysis of a large cohort of IM treated CML patients in ECP, has been proposed by the European LeukemiaNet (ELN); the variables included are percentage of basophils and spleen size.

Methods

The patients were enrolled in two phase 2 studies conducted by the GIMEMA CML WP (ClinicalTrials.gov. NCT00481052 and NCT00769227) or were treated with NIL as initial treatment in Bologna. Definitions: complete cytogenetic response (CCyR) was defined as the absence of Ph+ metaphases over at least 20 metaphases examined by conventional banding analysis or <1% BCR-ABL+ nuclei over 200 nuclei examined by FISH; major molecular response (MMR) was defined as BCR-ABL<0.1%; failure was defined according to 2009 ELN criteria; progression was defined as the transformation to accelerated or blast phase. All the calculations were performed according to the intention to treat principle.

Results and Significance

215 patients were included; median age 53 years (range 18-86). The patient distribution according the different scoring systems was as follows: 95% low and 5% high EUTOS score; 38% low, 44% intermediate and 18% high Sokal score; 39% low, 56% intermediate and 5% high Euro score. The median follow up was 29 months (range: 18-43 months). The cumulative CCyR rate was 93%; the cumulative MMR rate was 89%; the failure free survival was 90%; the progression free survival 93% and the overall survival was 94%. No difference in the cumulative CCyR rate at any time was observed according to EUTOS score, but patients with low EUTOS score achieved a significantly higher cumulative rate of MMR (91% versus 60%, p=0.01). Interestingly, the patients with low EUTOS score had higher failure survival (91% versus 70%, p=0.02), higher progression free survival (94% versus 80%, p=0.05) and higher overall survival (95% versus 79%, p=0.04). The Sokal score was able to predict differences in terms of MMR at any time and failure free survival, but not in terms of progression free survival or overall survival. The Euro score failed to detect any response and outcome difference.

Conclusions

In a cohort of CML patients treated with NIL-based regimens as front-line therapy, the prognostic predictive ability of EUTOS score resulted superior to Sokal and Euro score.

[5] The E13A2 BCR-ABL1 Fusion Transcript Is a Candidate Adverse Prognostic Factor in Chronic Myeloid Leukemia Patients Treated Frontline with Imatinib Mesylate

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Objectives and Background

The aim of the study was to assess the impact of the e13a2 (b2a2) or the e14a2 (b3a2) fusion transcripts on the long term outcome of early chronic phase (CP) chronic myeloid leukemia (CML) patients treated frontline with imatinib mesylate (IM). The e13a2 and the e14a2 are the most frequent BCR-ABL1 transcripts in CML. Few data about their prognostic value are available, particularly in early CP setting. Two small studies reported superior cytogenetic and molecular responses in patients with an e13a2 transcript (de Lemos JA, Genet Mol Res 2005; Sharma P, Ann Hematol 2010). Another experience failed to detect any response or outcome differenc...

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Objective and background
This case of Ph- non Hodgkin lymphoma occurring in a Ph+ CP-CML patient is the first report in the imatinib era, thus suggesting the rarity of association of myeloid and lymphoid neoplasms and multi pathway advancement of cancer cell survival against normal cells. Despite the origin of Ph negative hematopoietic clones still being unclear, their occurrence at diagnosis or after chemotherapy as well as after interferon alpha (IFN-α) or tyrosine kinase inhibitor (TKI) treatments argues against a therapy related event and it might suggest multi step process of CML pathogenesis or an intrinsic defect of hematopoietic stem cell or of immunity surveillance.

Case report
A 55 y old man with a diagnosis of Ph positive chronic myeloid leukemia in chronic phase (CP CML), low risk category according to Sokal score (haemoglobin 12.2 g/dl, platelets 292x10^9/L, white blood cells 75.5x10^9/L, spleen at the costal margin) was admitted at our hospital in May 2010. Imatinib treatment at the standard dose was started in June 2010 following a short initial cytoreduction with hydroxyurea. Despite optimal response to treatment being achieved within 3 months of imatinib treatment, peripheral lymphocytosis was documented since September 2010. At 6 months of treatment, significant (<2log) reduction of BCR-ABL ratio was measured by molecular test on peripheral blood, bone marrow dry tap hindered monitoring of imatinib efficacy, and bone marrow biopsy revealed erythroid hyperplasia with myeloid and megakaryocytic hypoplasia. At 10 months of treatment, major molecular response (MMR) was achieved, lymphocytes with aberrant phenotype and with clonal complex karyotype were detected in peripheral blood (phenotype of peripheral lymphoid blood cells: CD3+ CD2+ CD5+ CD7+ CD4- CD8- HLA-DR- TCRg/d-CD34-CD30-CD113-; conventional cytogenetic analysis on in vitro cultured lymphocytes: 46, XY, t(X;14)(q28;q11), -7,del (12) (p11.2),del(20)(q11.2),+mar(20); peripheral blood PCR: negative monoclonal gamma-T-cell receptor gene rearrangement). At 12 months of treatment, bone marrow biopsy revealed small lymphocytic infiltration (10%) predominantly composed of CD34 negative T cell with a monoclonal gamma-T-cell receptor gene rearrangement (histology and PCR tests). At the same time point total body CT scan showed multiple enlarged lymph nodes in laterocervical, inguinal, axillary, retroperitoneal areas (max diameter 28mm) that lead to diagnosis of peripheral T cell Lymphoma (PTCL), not otherwise specified (NOS) by axillary lymph node biopsy analysis. Thereafter chemotherapy was administered while MMR was confirmed on peripheral blood: complete hematological and cytogenetic response of lymphoproliferative disease was reached only after a third line approach involving administration of immunotherapy (firstly CHOP like scheme, secondly IGEV scheme and in the end treatment with Mab-Campath and Gemcitabine). Imatinib treatment was discontinued following 18months of administration due to chemotherapy and lymphoproliferative disease related myelosuppression and MMR of LMC was confirmed thereafter every 6 months. At the present time, the patient has undergone a haploidentical stem cell transplant due the availability of familiar donor.

Conclusion
While the biological and clinical significances of these other chromosomal abnormalities in Ph- cells of Ph+ CML patients require further investigation that might lead to treatment improvement, regular clinical and cytogenetic follow up of all patients receiving imatinib remains imperative.

[7] Long-Term Outcome of Chronic Myeloid Leukemia Patients Treated Frontline with Nilotinib 400 MG BID: 5-Year Update of the GIMEMA Trial CML0307

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Results and Significance
The median follow-up was 75 months; 95% of the patients had at least a 5 year observation. The CCyR rate at 12 months was not significantly different (75% and 79% in e13a2 e14a2 patients, respectively, p=0.274), but the MMR rate at 18 months and the MR4.0 at 36 months were significantly lower in e13a2 patients (52% and 67%, p=0.001; 20% and 30%, p=0.003, respectively). The times to MMR and MR4 were significantly longer for patients with e13a2 transcript (6 and 12 months, p<0.001; 37 and 54 months, p=0.002, respectively). The overall survival (p=0.023), the progression free survival (p=0.007), the failure free survival (p=0.045) and the event-free survival (p=0.012) were significantly lower in patients with e13a2 transcript. The outcome differences between e13a2 and e14a2 patients were confirmed by a multivariate analysis (Cox model).

Conclusion
The e13a2 transcript is a candidate adverse prognostic factor in early CP CML treated frontline with IM. An independent evaluation on in vitro cultured lymphocytes: 46, XY, t(X;14)(q28;q11), -7,del (12) (p11.2),del(20)(q11.2),+mar(20); peripheral blood PCR: negative monoclonal gamma-T-cell receptor gene rearrangement). At 12 months of treatment, bone marrow biopsy revealed small lymphocytic infiltration (10%) predominantly composed of CD34 negative T cell with a monoclonal gamma-T-cell receptor gene rearrangement (histology and PCR tests). At the same time point total body CT scan showed multiple enlarged lymph nodes in laterocervical, inguinal, axillary, retroperitoneal areas (max diameter 28mm) that lead to diagnosis of peripheral T cell Lymphoma (PTCL), not otherwise specified (NOS) by axillary lymph node biopsy analysis.

Results and Significance
73 patients enrolled; median age 51 years; 45% low, 41% intermediate and 14% high Sokal risk. The cumulative incidence of CCyR was 100%. Only 1 patient had a confirmed loss of CCyR and subsequently progressed to blast phase (BP). Two out of 73 patients never achieved a MMR: one progressed to BP, the other one still on stable and confirmed CCyR at 48 months. Only 3 patients had a confirmed loss of MMR due to low adherence and are still on nilotinib. The overall estimated probability of MR4.0 was 82%; 25% (18/73 patients) showed a stable MR4.0. Only one patient progressed at 6 months to lymphoid BP and subsequently died (high Sokal risk, BID as frontline therapy of CML (ClinicalTrials.gov: NCT00481052). The median follow-up for the present analysis was 51 months (range: 48 - 58 months); results with 5-year median observation will be presented on site. Definitions: major molecular response (MMR), BCR-ABL/ABL ratio <0.1%; MR4.0, BCR-ABL/ABL ratio <0.01 % IS; stable MR4.0, at least 3 consecutive samples; failure, according to 2009 ELN recommendations; event, failure or treatment discontinuation for any reason. All the analysis has been made according to the intention-to-treat principle.

Results and Significance
73 patients enrolled; median age 51 years; 45% low, 41% intermediate and 14% high Sokal risk. The cumulative incidence of CCyR was 100%. Only 1 patient had a confirmed loss of CCyR and subsequently progressed to blast phase (BP). Two out of 73 patients never achieved a MMR: one progressed to BP, the other one still on stable and confirmed CCyR at 48 months. Only 3 patients had a confirmed loss of MMR due to low adherence and are still on nilotinib. The overall estimated probability of MR4.0 was 82%; 25% (18/73 patients) showed a stable MR4.0. Only one patient progressed at 6 months to lymphoid BP and subsequently died (high Sokal risk,
T315I mutation). Overall, 11 patients (15%) discontinued nilotinib permanently: 1 progression to BP; 3 recurrent myelosse and/or lipase increase (no pancreatitis); 3 peripheral arterial obstructive disease (age at nilotinib start: 65, 66 and 76 years; 2 out of 3 with at least 1 cardiovascular risk factor); 1 atrial fibrillation (unrelated to study drug); 1 death through mental deterioration and starvation (unrelated to study drug); 2 patient refusals (1 patient still off-treatment, with stable MR4 after 9 months; 1 patient on imatinib). The 5-year estimated probability of overall survival, PFS and failure-free survival was 97%; the 5-year estimated probability of EFS was 83%.

Conclusions
The great majority of patients are still on nilotinib. Only 1 patient progressed to advanced phase so far: considering the yearly transformation rate with any TKI in CML (most progressions were reported during the first 2-3 years with nilotinib and during the first 1-2 years with nilotinib and dasatinib), a relevant transformation incidence in the next years is very unlikely. Given the very high rate of deep molecular response, many patients are candidate to treatment discontinuation.

[8] Long-Term Persistence of Molecular Remission after Imatinib Discontinuation
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Objective
To explore the 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 44 non-selected patients with CML in early chronic phase who were receiving imatinib in routine practice. Recommended dose of imatinib at the start of treatment were 400 mg daily. We have analyzed the correlation between grade of cytogenetic response after 18 months of imatinib treatment, at time points of 6, 12 and 18 months correspondently, and median daily dose (MDD), calculated as actual number of capsules taken reported by patients. After every 6 months dose escalation up to 600 mg or 800 mg daily could be recommended based on monitoring findings.

Results
In the group of 44 pts with CML in early chronic phase the observed MDD in the first 6 months of therapy was 317.8 mg daily. In the sub-group of 30 pts of 44, MDD was higher – 372.5 mg daily. In these patients we saw major cytogenetic response in the majority of cases, 22 of 30 (73.3%). Nine pts (30%) reached complete cytogenetic response (CCR), 13 pts (43.3%) – partial cytogenetic response (PCR), 6 pts (20%) – minimal cytogenetic response (MCR). Only 2 in 30 (6.7%) did not reach cytogenetic response. In the other subgroup of 14 pts of the 44, MDD was lower – 200.5 mg daily. In this subgroup MCR was registered in 35.7 % of cases and there were no cytogenetic response in 63.6% of cases. 19 pts were recommended to escalate the dose of imatinib up to 600 mg daily. Instead of intention to treat, actual median dose by 12 months was 425.2 mg daily. 28 pts received MDD 517.6 mg. In this subgroup with MDD higher than 317.8 mg daily 11 (63.6%) pts reached major cytogenetic response (CCR - 18.2%, PCR – 45.4%), 4 patients reached MCR or did not reach a response. There was MDD less than 425.2 mg – 369.7 mg in the second subgroup of 17 pts. Major cytogene-
[10] Early Predictors of Progression to Accelerated-Blastic Phase in Patients with Chronic Phase Chronic Myeloid Leukemia Treated with Nilotinib Frontline

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Objectives and Background
Nilotinib is a second-generation tyrosine kinase inhibitor (TKI) that has demonstrated higher and faster responses and lower progression rates compared to imatinib in early chronic phase chronic myeloid leukemia patients (ECM CML). Most progression to accelerated/blastic phase (AP/BP) in patients treated with TKIs occurs within the first 1–2 years of treatment. The identification of early factors that may predict for progression represents an important issue in order to optimize the treatment. Early molecular responses have been associated with a better progression-free survival in patients treated with nilotinib or dasatinib frontline, within company-driven studies. Here we analyse early factors that may predict for progression in an independent cohort of patients treated with nilotinib based regimens in Italy.

Methods
Two hundred fifteen patients were enrolled in two multicenter phase 2 studies conducted by the GIMEMA CML WP (ClinicalTrials.gov. NCT00481052 and NCT00769327) or were treated at the Dpt. of Hematology and Oncology, Bologna University Hospital, with nilotinib 300 mg BID or 400 mg BID as initial treatment. The median age was 53 years (range 18-86). The median follow-up was 29 months (range: 18–47 months). Definitions: complete cytogenetic response (CCyR) was defined as the absence of Ph metaphases over at least 20 bone marrow metaphases examined by conventional banding analysis or ≤1% BCR-ABL nuclei in peripheral blood by FISH ≥200 nuclei. I-FISH: major molecular response (MMR) was defined as the BCR-ABL/ABL ratio according to IS; progression was defined as the transformation to AP/BP.

Results
Overall, 9/215 (4.2%) patients progressed: 8/9 progressed occurring during the first year (2 patients at 4 months, 2 at 6 months, 2 at 10 months, 2 at 12 months and 1 at 25 months). At diagnosis 2 patients had clonal chromosome abnormalities; 2/9 patients had high EUTOS scores, 7/9 intermediate (3) and high (4) Sokal score, 6/9 intermediate EURO score. At the time of progression the ABL mutational status was: 6 wild-type, 4 T315I, 1 Y253H. In order to identify early predictors of progression to AP/BP we analysed the cytogenetic and molecular response rates at 3 months in patients with and without subsequent progression. At 3 months 212/215 (99%) of the patients were on study (2 early adverse events, 1 refusal); 189/212 (89%) and 196/212 (92%) were evaluable for cytogenetic and molecular responses, respectively. Nine-month follow-up was performed on 189/212 (88%) of the patients obtained a MCgR at 3 months, 4/159 progressed (2.5%); on the other hand, 7% (13/189) of the patients did not reach a MCgR at 3 months, 3/13 (23%) progressed (p=0.01). No significant statistical difference (p=0.08) was observed if CCyR vs. less than CCyR is considered (data not shown). Eighty-eight per cent (173/196) of the patients obtained a BCR-ABL/ABL ratio < 1% at 3 months, 5/173 (2.9%) progressed; on the other hand, 12% (23/196) of the patients had a BCR-ABL/ABL ratio at 3 months > 1%, 4/23 (17.4%) progressed (p=0.012).

Conclusions
In patients treated frontline with nilotinib-based regimens a MCgR and a BCR-ABL/RATIO < 1% at 3 months correlated in univariate analysis with significantly reduced rates of progression to AP/BP.

[11] Second Malignancies in Chronic Myeloid Leukemia (CML) Patients Treated Frontline with Imatinib – A Survey by the GIMEMA CML WP

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Objectives and Background
Imatinib (IM) is the standard of care for CML in early chronic phase, and presently remains a life-saving drug to be taken chronically. Long-term side effects, including the incidence of second (II) malignancies, represent a potentially relevant issue. Results from epidemiological studies indicated CML patients were at higher risk of developing II malignancies (Frederiksen et al., Blood 2011; Reborra et al., Am J Epidemiol 2010). Roy et al. (Leukemia 2005) reported an unexpec ted incidence of II neoplasms, and in particular of prostate cancers, in patients treated with IM after interferon; an increased incidence of prostate cancer was also reported in the ILTE study (Gambacorti-Passerini et al. – ASH Meeting 2011; abs. 3766). On the other hand, different studies (Pilot et al., Leukemia 2006; Ver ma et al., Blood 2011) did not provide evidence for an increased incidence of II malignancies. According to some in vitro studies, IM may enhance the malignant behaviour of some types of carcinoma cell lines. We analysed the incidence of II malignancies in a homogeneous cohort of patients treated frontline with imatinib and with a long follow-up.

Methods
Overall, 559 patients have been enrolled in 3 concurrent clinical studies of the GIMEMA CML Working Party with IM frontline (clinicaltrials.gov no. NCT00510926 and NCT00514488; the observational trial CML 023). To better define the incidence of II malignancies, in addition to the cases notified as severe adverse events, a specific query was sent to all participating GIMEMA Clinical Centres. Non-melanoma skin cancers were excluded. Malignancies prior to the diagnosis of CML were investigated too.

>> 12
Results
The requested data were obtained for 514/559 (92%) patients (52/62, 84% of the Centres). The median age at the diagnosis of CML was 52 (extremes 18 – 84) years. The median follow-up was 68 (2-91) months; cumulative patient years at risk were 2721. Twenty-nine patients (5.6%) developed a II neoplasm at a median time of 27 months (extremes 3-61) from the start of IM therapy. Six of these malignancies were diagnosed within 1 year. The median age at the diagnosis of II malignancy was 63 y (extremes 38-81). Five patients had also a diagnosis of malignancy prior to the diagnosis of CML. Overall, 15 out of 559 (2.7%) patients died due to II neoplasm progression, which represented the second cause of mortality after progression to accelerated-blastic phase. The comparison with the general population showed an increased incidence of II malignancy (SIR 1.6, CI 95% 1.12 – 2.12) and an increased mortality (SIR 3.06, CI 95% 2.15 – 3.98) in our cohort of patients.

Conclusions
In this multicentre nation-wide experience of CML patients treated with IM frontline, the incidence of II malignancies was superior to the expected incidence of neoplasms in the Italian national population. If the increased incidence of II malignancies reflects an intrinsic characteristic of the CML patients, unmasked by their increased survival, or it is favoured by imatinib treatment requires further investigations.

[13] Age Dependent Analysis of CML in Patients of the CML IV Study

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Introduction
The impact of age at diagnosis of CML on the course of the disease and the outcome has changed since the introduction of imatinib. We therefore sought to analyse different age groups for differences in manifestations and course of the disease.

Methods
From July 2002 until March 2012, 1551 patients with BCR-ABL positive CML in chronic phase were randomised to the CML study IV, a 5-arm trial of imatinib 400 mg vs. imatinib 800 mg vs. imatinib 400 mg in combination with interferon alpha vs. imatinib 400 mg after interferon failure. 1517 patients were evaluable. The median follow-up was 67.5 months. We analysed the differences in initial clinical presentation (fatigue, symptoms of organomegaly, spleen size, WBC counts, percentage of blasts, eosinophils, basophils, monocytes, platelets, risk profile, additional chromosomal aberrations and transcript types) and the differences in the response rates, dividing the patients of all therapy arms into four age groups: 118-29, 29-30-45, 30-45-60, >60 years of age.

Results
12 patients belonged to group 1, 384 patients to group 2, 495 to group 3 and 526 to group 4. In all four age groups there was a prevalence of male gender, but group 4 showed a higher percentage of female patients in comparison to group 1 (46% vs. 33%). Most patients had Karyological index >85%, but 12% of patients in group 1 had a Karyological index between

[14] Molecular Response in Patients with Chronic Myeloid Leukemia in Chronic Phase on Long Term Imatinib Treatment

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Background
Imatinib in chronic myeloid leukemia (CML) opened a new era in successful treatment. Imatinib achieved not only complete cytogenetic response but also good molecular response which was not possible before. In patients with complete cytogenetic response (CCyR), real-time reverse transcriptase PCR testing (RQ-PCR) is the method of choice for further monitoring of residual disease.

Aim: We intended to analyze the type of molecular response in a cohort of CML patients on long term frontline treatment with imatinib, lasting at least 3 years in Clinic of Hematology, Clinical Center of Serbia. Regular follow up was performed according to current proposals (ELN based national guidelines).

Patients and methods
62 CML patients, 33 males and 29 females, aged 20-79 yrs, treated for more than 3 yrs, were analyzed by RQ-PCR during first six months of 2012. 59 pts were on 400 mg/d and 3 pts were on 800 mg/d of imatinib. Cytogenetic analysis was performed on bone marrow cells by HG-band technique. At least 20 metaphases were analyzed after preparation of bone marrow specimens directly or after short-term cultures (24h). Molecular monitoring was assessed by RQ-PCR according to EAC protocol on ABI 7500 Real Time PCR System in peripheral blood samples. For absolute quantification, standards of the BCR-ABL and ABL genes (Ipsogene) were used and the BCR-ABL/ABL ratio was compared according to baseline (BCR-ABL/ABL ratio in 30 day novo CML patients). IS conversion factor is pending in near future. The level of BCR-ABL/ABL ratio was assessed according to recently published data (Cross, Leukemia 2012).

Conclusions
Imatinib can achieve good molecular response in the long term. Our results show that a significant proportion of patients can be candidates for future discontinuation trials but also many of them may need stronger kinase inhibition before such trials. On the other hand, incorporation of molecular monitoring into regular follow up is necessary for early prediction of undesirable events like loss of response.
Inhibition of Hedgehog Signalling in Human Acute Lymphoblastic Leukaemia (ALL)

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50-80 %, 9% in group 2 and 3 each and 16% in group 4. Group 1 presented with a larger spleen size (median length 5 cm below the costal mar- gin), whereas groups 2, 3 and 4 had a median length of 3 cm (p=0.002), 1cm (p<0.001) and 0 cm (p<0.001), respectively, and presented more often with symptoms of organomegaly (28%) in comparison to groups 2, 3 and 4 (24%, 18%, and 7%, respectively).

Group 1 presented with a higher number of WBC (median 144 x 10^9/L) than group 2 (106 x 10^9/L p=0.048), group 3 (74 x 10^9/L p<0.001) and group 4 (57 x 10^9/L p< 0.001) and also with a higher percentage of blasts in the peripher- al blood (but not in the bone marrow), median 2 % in comparison to 1%, 1% and 0% in groups 2, 3, 4, respectively. Group 1 presented with lower haemoglobin levels with a median of 11.1 g/dL compared to 11.8 g/dL (p=0.045), 12.6 g/dL (p<0.001) and 12.5 g/dL (p< 0.001) in groups 2, 3, and 4, respectively. 80% had a low risk EUTOS score in group 1 compared to 84% in group 2, 89% in group 3, and 92% in group 4. There were no differences in presence of fati- gue, percentage of eosinophils, basophils, and platelets, additional chromosomal aberrations and type of BCR-ABL transcripts at diagnosis between the four groups.

No significant differences were found in the rates of complete cytogenetic remission, major molecular remission and complete molecular remission during the observation time. 11 of the 112 patients (9.8%) of group 1, 29 of 384 patients of group 2 (7.5%), 25 of 495 (5%) of patients of group 3 and 31 of 526 (5.8%) of patients of group 4 progressed to accelerated phase or blast crisis. As expected, more patients in group 1 were transplanted (19%) compared to 12%, 7% and 1% in groups 2, 3 and 4.

Conclusions
Patients at 18-29 years of age appeared to pre- sent with a more aggressive disease than the other age groups, with higher levels of WBC and blasts, lower haemoglobin, larger spleen size and more frequent organomegaly related symptoms. This is reflected by a worse EUTOS. The initial aggressive manifestation of the disease in the younger age group did not correlate with lower rates of major molecular remission and complete molecular remission although the rates of progression to accelera- ted phase or blast crisis were higher in group 1 than in the other groups, particularly groups 3 and 4.

Objectives and Background
The Hedgehog (Hh) pathway plays a func- tional role in embryonic development and oncogenesis. Modulation of Smo activation, an essential component of the Hh pathway, influences the stem cell number and disease acceleration in BCR-ABL positive CML by influencing self-renewal. Clinical trials us- ing Hh inhibitors have started in BCR-ABL pos ALL and CML. The role of Hh signalling on stem cell behaviour in BCR-ABL neg ALL has not yet been examined. The phenotype of leukemic stem cells (LSCs) and the target cells for transformation in ALL are contro- versial. They may be the most relevant tar- gets for new treatment regimens, provid- ing promising promises for improving treat- ment of adult ALL. Aims of the study are characterisation of Hh signalling inhibition in ALL, using twelve long term cultures of ALL blasts (LTCs), being collected at prima- ry diagnosis, focusing on leukemia initiat- ing and clonogenic capacity, proliferation, and apoptosis. In addition LSC function and cell fate decisions are being assessed using video microscopy-based single cell track- ing. These studies are anticipated to evalu- ate the therapeutic potential of Hh inhibi- tion and combination therapy in both BCR-ABL pos and neg ALL.

Methods
As models of BCR-ABL pos and neg leuke- mia we used serum-, cytokine- and stroma free long-term cultures of primary ALL blasts. Clonogenic growth of ALL cells was assessed in semi-solid methylcellulose based me- dia. Cell sub populations were isolated on the basis of CD20, CD34 and CD38 expression via FACS sorting. Cell proliferation and apop- tosis were measured by XTT assays and AnnexinV/7AAD-FACS stain- ing. For RT PCR of Hh signalling pathway components we used pre- developed Taqman assays. Single cell video tracking to determine cell fate decisions was performed as previously described (Rieger et al., Science 2009). Two Smo inhibitors currently in clinical test- ing, LDE225 and BMS839293 were kindly provided by Novartis and BMS, respectively.

Results and statistical significance
Most sorted sub populations are capable of reinitiating LTCs and revert to the original pre sort immunophenotype with the excep- tion of CD34+ cells in two LTCs. These sub populations maintained the marker- profile of the sorted cells for more than 90 days and displayed slower growth kinetics. The frequency of colony form- ing units ranged from 0.25% to 5%. Analysis of Hh Signalling in ALL LTCs by PCR demonstrated expression of Shh, Ptc, Smo, and the transcription factors GlI 1+3, indicating active Hh signalling in ALL. Interestingly Gli 2 was not expressed, the functional relevance of which remains as yet unclear. Hh inhibition showed no dose depen- dant effect on proliferation or apoptosis.

Conclusions
We found evidence of Hh activation in both BCR-ABL pos and neg LTC ALL cells. No impact of Smo inhibition on proliferation and apoptosis was observed in response to the Smo inhibitors LDE225 and BMS839293, consistent with the hypothesis that Hh signalling in these cells may affect primarily self renewal mechanisms. Single cell imaging of ALL LTCs has been successfully established for up to nine days of culture and will reflect the influence of Hh inhibi- tion on cell fate decisions.


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Introduction
Considerable improvements in the treat- ment of CML have been made during re- cent years. This has led to a point where the classical time-to-event endpoints like death and end of chronic phase can only be analy- sed meaningfully after an exceptional long observation time. For example, in the Ger- man CML Study IV first treatment compar- isons for overall survival are planned about fifteen years after start of the study. As a substitution, composite time-to-event end- points have become more and more popu- lar. From a statistical point of view, this has to be judged critically.

Methods
We analysed data from the German CML Study IV using the composite endpoint “Fail- ure free Survival” (FFS), which is defined as death, progression to accelerated phase/ blast crisis, less than complete hematolog- ic remission (CHR) at 3 months, less than complete cytogenetic re- mission (CCyR) at 18 months and loss of partial cytogenetic remis- sion (PCyR) after achieving a CCyR. By way of comparison, particu- lar endpoints were separately analysed using proportional hazard models and assuming the others as competing events (death, pro- gression and loss of PCyR). When analysing loss of PCyR, left trun- cation had to be taken into account. For achievement of CHR and CCyR at 3 respectively 18 months, odds ratios (OR) were calculated and landmark curves were estimated to assess the prognostic impact of this particular events on overall survival.

Results
Out of 1466 evaluable patients, 571 were available for FFS. When not demanding a hematologic investigation at 3 months, still only 726 patients (49.5%) were evaluable. 740 patients (50.5%) had no cytogenetic assessment at 18 months, even if they had been ob- served for more than 18 months. This may be due to the substitu- tion of cytogenetic by molecular assessments, which has become standard after the publication of the ELN recommendations, but still 467 patients (31.9%) had neither.

When comparing EUTOS high risk to low risk groups with respect to FFS, hazard ratio (HR) was 1.670, respectively 1.622 when omitting
the achievement of CHR at 3 months. For the particular endpoints, HRs were 0.900 (death without prior progression), 2.118 (progression to accelerated phase/blast crisis) and 1.642 (loss of PCR). ORs were 2.122 (CHR at 3 months) and 1.964 (CCyR at 18 months).

Using a landmark approach, achievement of CHR at 3 months had no significant impact on overall survival, in contrast to CCyR at 18 months (p<0.001).

Conclusions

Missing values are a serious problem, since every patient with one missing assessment cannot be considered for analysis. Furthermore, when comparing treatments, this may be in conflict with the intention-to-treat principle.

Analysing the particular endpoints separately has shown that there are considerable differences with respect to the HR and OR. The HR for FFS seems to be a sort of average of these values. Landmark analyses have shown that the severity of the particular endpoints is rather different, which makes interpretation of the composite endpoint difficult.

If possible, composite time to event endpoints should be avoided. Instead the particular events should be analysed separately using a competing risks approach.

Background

Success of tyrosine kinase inhibitors (TKIs) in CML has given pts hope for a long disease-free survival. The increase in survival can reveal long-term effects of TKI treatment such as the development of other tumours (second malignancies, SM). According to the literature, the incidence of SM varied from 1.02% (Kim DW, et al. Blood 2009) up to 4.02% (Verma D, et al. Blood 2008);

similar in terms of a cohort of pts analyzed by the research group GIMEMA (Gugliotta G, et al. Blood 2010).

Aim

To evaluate the incidence and variants of SM in CML patients treated with imatinib (IM) frontline in clinical practice in Russia.

Methods

Data were analyzed from 29 administrative districts of Russia. The selection of regions was based on the quality of the data from CML pts registry. The study involved 607 CML pts, but in the analysis were included 601 pts without a history of cancer in anamnesis (6 pts with a primary non-hematologic tumor in anamnesis) with the criteria (EUTOS OSP-study): Ph/BCR-ABL-positive CML diagnosed in 2002-2006, age of pts ≥ 18 years (y), initiation of IM therapy ≤ 6 months (mo) from the date of diagnosis.

Median (Me) age was 48 (18-82) y, sex ratio (M/F) 47/53% pts. Median age at the diagnosis of CML was 59 (44 – 75) y; Median time for detection of SM since diagnosis of CML was 37 (14-57) mo. Median time of IM therapy 58 (14-71) mo. Three-year overall survival for patients with SM was 64% (Me 20 mo, extr.2.8-58.9).

The most common SM: breast cancer (3 of 11 cases) and tumour of the gastrointestinal tract (3 of 11 cases). Most of the pts had good results of TKI therapy: 5 of 11 pts achieved major (MMR) and complete molecular responses (Median time 56 (range 6-67) mo), 3 out of 11 pts- CCyR.

Conclusion

For the first time we present the frequency of cases in CML with the Russian regions, the study OSP EUTOS. Mortality from all causes in a cohort of 601 pts was 15.1% (91 pts), of which 4.4% (4 pts) because of progression of SM.

Results

The cumulative incidence of SM in our CML pts was 1.82% (11 pts). The probability of occurrence SM at 5 years after CML diagnosis was 0.0192 (95%CI 0.0116-0.0375) (fig.1). Sex ratio (M/F) 4/7 pts. Median age at the diagnosis of CML was 59 (44 – 75) y; Median time for detection of SM since diagnosis of CML was 37 (14-57) mo. Median time of IM therapy 58 (14-71) mo. Three-year overall survival for patients with SM was 64% (Me 20 mo, extr.2.8-58.9).

The most common SM: breast cancer (3 of 11 cases) and tumour of the gastrointestinal tract (3 of 11 cases). Most of the pts had good results of TKI therapy: 5 of 11 pts achieved major (MMR) and complete molecular responses (Median time 56 (range 6-67) mo), 3 out of 11 pts- CCyR. Mortality in a cohort of 601 pts was 15.1% (91 pts), of which 4.4% (4 pts) because of progression of SM.

[16] Second Malignancies (SM) in Patients (PTS) with Chronic Myeloid Leukemia (CML) in Clinical Practice Analysed in the Frame of International Research Project EUTOS OSP in the Russian Federation (RUS).


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Conclusions

Missing values are a serious problem, since every patient with one missing assessment cannot be considered for analysis. Furthermore, when comparing treatments, this may be in conflict with the intention-to-treat principle.

Analysing the particular endpoints separately has shown that there are considerable differences with respect to the HR and OR. The HR for FFS seems to be a sort of average of these values. Landmark analyses have shown that the severity of the particular endpoints is rather different, which makes interpretation of the composite endpoint difficult.

If possible, composite time to event endpoints should be avoided. Instead the particular events should be analysed separately using a competing risks approach.
[17] Spectrum of BCR-ABL Gene Mutations in Resistant CML Patients Monitored at One Centre

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Background
Treatment resistance remains an important issue in chronic myelogenous leukemia (CML) despite a dramatic improvement of its treatment after the introduction of tyrosine kinase inhibitors (TKi). BCR-ABL gene mutations are among the most studied and significant causes of TKi resistance; however, their role is still a subject of debate.

Methods
A group of 29 CML patients (18 men and 11 women) were investigated for BCR-ABL gene mutations, “GenoTechnologija” laboratory, Russian Federation. Four were investigated at initial diagnosis (3 – chronic phase; 1 – blast crisis), one was investigated after interferon alfa (IFN) treatment failure, and the remaining patients were investigated under treatment with imatinib (IM), either as front-line (n=5), 2nd line or 3rd line treatment.

Results
BCR-ABL mutations were detected in 9 previously treated patients (31%). Among subjects investigated before initiation of IM: one case with T83T mutation was revealed while the other 3 cases had no mutation. Of 9 previously treated patients who had mutations – 6 subjects were pre-treated with IFN for an average of 15.6 months before IM start, with average overall disease duration was 62.4 months at the time of mutation analysis. The remaining 3 were pre-treated with hydroxyurea for an average of 11.5 months before IM start, with average overall disease duration of 40 months. The profile of detected mutations was as follows: E255K/V – in 3 patients (33%), F359V – in 2 patients (22%), mutations M351T, T315I, Y253H, G250E – in one patient each. No mutations were detected in 2 patients (22%); mutations M351T, T315I, E255K/V – in 3 patients (33%), F359V – in 2 patients (22%), mutations M351T, T315I, Y253H, G250E – in one patient each. Notably, T315I mutation was revealed in a patient treated with IM front-line who had a T83T mutation before treatment. Two patients with E255K/V and one patient with Y253H belonged to high Sokal and Hasford risk group. Intermediate risk was confirmed in 2 patients with F359V and 1 with E255K/V at diagnosis. Patients with M351T, G250E, and T315I belonged to the low risk group.

Conclusions
All patients with BCR-ABL mutations as well as 6 patients without mutations were treated with nilotinib 800 mg daily. As a result – 4 patients including 2 with E255K and 2 without mutations achieved only CHR without cytogenetic response. Two of them (one patient bearing E255K and a patient without it) lost their CHR after 3 and 6 months respectively. Partial cytogenetic response was achieved in a patient with G250E. Complete cytogenetic response was reached in 3 patients including one with F359V detected before nilotinib. Interestingly, the latter patient achieved elimination of mutated clone and complete molecular response within a year of nilotinib treatment despite poor sensitivity reported for this mutation to nilotinib. The remaining 5 patients with E255K, M351T, T315I, Y253H and F359V achieved neither cytogenetic nor hematological response and stopped nilotinib.

[18] Results of the CML Andalusian Registry (RALMC).
Current Status of 162 Ph+ CML Patients According to the European LeukemiaNet (ELN) Guidelines 2009 and to the Provisional Definition for Second-Generation TKIs (TKI2G) as First-Line Treatment.

Andalusian CML Group

Background
Andalusia is a Spanish region with 8.5 million people, a rate of 80 CML diagnoses per year and an estimated prevalence of 600 patients. The RALMC was created by the CML Andalusian Group in 2005 and aimed at examining CML in Andalusia. It was revised in 2012 with an on-line CRF adapted to the 2009 ELN guidelines.

Objectives
1. To know the burden of CML in Andalusia: incidence, prevalence, mortality, and survival (overall, event free and progression free) and by age and gender. To describe epidemiology, clinical characteristics, diagnostic and treatment of CML patients in the RALMC.
2. To endorse the RALMC as a useful tool for the control of CML.

Methods
Descriptive retrospective study of CML patients based on an online CRF. Data are introduced by designated medical staff at each center and supervised by a coordinator. All hospitals in Andalusia can take part in the study.

Results
429 patients from 35 hospitals. Data are updated every 3 or 6 months. By the end of 2012, the registry is expected to contain 600 patients. Median age is 54, gender distribution: 52% male and 48% female, distribution by Sokal groups: low risk 41%, intermediate risk 39% and high risk 20%. Only 10 cases were diagnosed in accelerated phase. TKI2G were used as first-line therapy.
in 20 patients. We performed a sub-analysis on data from 142 patients classified following the 2009 ELN guidelines, and from 20 patients treated with TKI2G classified following the provisional definition: 142 patients were on Imatinib 400 QD: 106 (74.6%) had optimal response (48 MR 5.0; 30 MR 4.5; 13 MR 4.0; 11 MMR; 3 CCyR; 1 CHR), 16 (11.4%) suboptimal response; 10 (7%) failure and 10 (7%) intolerant. 36 patients were on second-line therapy: 23 on Dasatinib 100 QD: 15 (63%) optimal response (4 MR 5.0; 7 MR 4.5; 1 MR 4.0; 3 MMR); 1 suboptimal response; 4 failure and 3 intolerant. 13 on Nilotinib 400 BID: 9 (69.2%) optimal response (5 MR 5.0; 2 MR 4.5; 2 MMR); 1 suboptimal response; 3 intolerant. On third-line therapy: 1 patient was on Dasatinib (MR 4.5) and 3 on Nilotinib (MR 4.0).

All 20 patients treated with TKI2G as first-line (14 Nilotinib, 6 Dasatinib) reached CHR at month 1. 14 and all were evaluated at month 3; all of them had BCR-ABL < 10%. At month 6, 10 patients were evaluated; all of them had CCyR, 6 MR 4.5, 1 MMR and 3 had no MMR (BCR-ABL= 0.15, 0.2 and 0.7%). No noteworthy adverse events were reported, and drug administration was not discontinued in any case.

Conclusions

RALMC is a useful tool to dimension CML in Andalusia. Using this application in clinical practice will improve the quality of patients’ care, and will allow the extrapolation of epidemiological studies. Although Imatinib has proven its efficacy in our series, with a similar turn-out to those in the literature, with Nilotinib and Dasatinib, we have achieved BCR-ABL percentages under 10% in all cases, which constitute an independent factor to global survival, according to the latest publications.

[19] Functional Analysis of Bone Marrow (BM) Niches for the Regulation of Tyrosine Kinase Inhibitor (TKI) Activity on Philadelphia Positive (Ph+) Cell Lines and CD34+ Progenitor Cells Derived from Patients with CML

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Introduction

The importance of tumour microenvironment for cancer progression is becoming widely recognized in recent years. The Bone Marrow (BM) is a dynamic microenvironment with a high concentration of growth factors and cytokines necessary for hematopoiesis, making it a highly permissive zone for cancer hematopoietic stem cell homing and survival. It is possible that the same factors that modulate hematopoiesis promote leukemogenesis, enhance blast survival and make them resistant to treatment within the BM microenvironment. In the era of targeted molecular therapy, where Imatinib has shown a cumulative best complete cytogenetic response rate of 82% and an estimated event free survival at 8 years of 85%, several in vitro data have confirmed that Ph+ CD34+ progenitor cells crammed in BM niches are resistant to TKI treatments. We attempted to define BM microenvironment markers that nurture and determine stem cell fate in leukemia associated niches.

Methods

We treated Ph+ K562 cell lines and primary CD34+ BM cells derived from untreated CML patients with a dose range of TKIs (0-100µM) in the presence of a monolayer of human BM mesenchymal stromal cell line (HS-5) or HSS conditioned media (HCM), to assess the role of BM niches in the regulation of TKI responsiveness. Selected experiments were conducted by adding several cytokines, known to be critical mediators of stromal/leukemic cell interaction, to the in vitro culture.

Results

We demonstrated that the BM stroma environment significantly protects the K562 cell line from TKI-induced apoptosis. Indeed, we demonstrated that IC50 value (calculated either on proliferation or apoptotic assay) of Imatinib, Nilotinib and Dasatinib increased by almost one log when leukemic cells are exposed to HSS or HCM. We detected more than 60% of proliferating cells and less than 40% of apoptotic cells when the K562 cell line was observed after treatment with 300 nM Imatinib, 30 nM Nilotinib or 3 nM Dasatinib in the presence of HSS or HCM. Interestingly, we demonstrated that TKI treatment is not able to reduce STAT3p when the K562 cell line is also exposed to HSS cell line. Thus, we evaluate if the major stromal-derived cytokines, i.e. SDF-1, SCF, IL3, IL6, IL8, G-CSF and GM-CSF might be responsible for the regulation of TKI responsiveness in Ph+ cell line treated with TKIs. We demonstrated that the applied cytokines did not significantly modify TKI-induced apoptosis in either K562 or CD34+ primary CML cells. Thus, we are currently screening with a multi-parametric approach all known soluble factors secreted by HS-S cell line.

Conclusions

Taken together, these findings indicate that BM-derived stroma cell line produces a strong effect on the regulation of TKI responsiveness in Ph+ CML cells by both a direct cell-to-cell contact and exposition to soluble factors.

[20] Clinical Relevance in Chronic Myeloid Leukemia of Deletion and Insertion Events in the Tyrosine Kinase Domain of BCR-ABL


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Background

While the great majority of CML patients achieve a deep response to the treatment with inhibition of TK, some show only suboptimal response (SO) or resistance to TKI treatment. One of the most frequent causes of resistance is the onset of point mutations at the catalytic site of ABL. These mutations impair interaction of oncogenic BCR-ABL protein to Imatinib and, at least extent, also to second generation TKI. We characterized the ABL deletions/insertions in 23 CML patients who showed Imatinib resistance.

Methods

A total of 830 patients were treated with Imatinib and monitored by RTQ-PCR, according to ELN recommendations. In 225 suboptimal or failure patients the ABL sequence of BCR-ABL was analyzed and deletions/insertions were confirmed by ARMS-PCR. Based on wild type BCR-ABL crystallography, mutated sequences were analyzed by homology modelling (HM) to generate 3D structures and to predict TKI bindings.

Results

ABL mutations were detected in 118 out of 225 (52%) tested patients. 80% of them reported single amino acid substitutions and the remaining patients showed ex7-9 ABL deletion (17%) or insertion (3%). No differences were observed in terms of age (median 52 ±18, range of 26-78), sex and WBC count between patients showing point mutations or showing deletion/insertion events.

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Objectives and background
Advances in CML treatment substantially prolong patients’ lives. Cytogenetic monitoring has revealed that about 6-10% of patients have additional chromosome abnormalities (ACA) in Philadelphia-positive (Ph+) and Philadelphia-negative (Ph-) cells. Recently several reviews have published evidence of a negative prognostic role of ACA in Ph+ cells, but the value of ACA in Ph- cells has not yet been established. The aim of our study was to assess the significance of ACA in Ph- cells in CML patients.

Methods
We observed 10 cases of patients with ACA in Ph- cells in CML diagnosed since 2005 (when imatinib became the first-line treatment standard in Russia). The control group included 104 chronic phase CML patients without ACA in Ph- cells. Conventional cytogenetic approaches were used to monitor treatment response at 3, 6 months, 1 year of treatment and half-yearly thereafter until Complete Cytogenetic Response (CCyR) was obtained. Cytogenetic analysis was also performed in cases of BCR-ABL elevation, loss of hematologic response or unexplained cytopenias. We assessed overall survival (OS), event-free (death or progression to accelerated or blast phase) survival (EFS), probability to reach CCyR and Major Molecular Response (MMR) in patients that have ACA in Ph- cells compared to the control group. Statistical analysis included Kaplan-Meyer analysis with Log-rank test for survival comparisons and Chi-square tests for categorical variables.

Results
Among 10 cases with ACA in Ph- cells we identified 5 cases with del7, 4 cases with +8 and 1 case with del11. Median time from diagnosis to ACA in Ph- cells detection was 1.96 years (0.3-4.6 years). There were significant differences in OS (p=0.03) and EFS (p=0.05) with lower survival for patients with ACA in Ph- cells. Probabilities to obtain CCyR and MMR were significantly lower in ACA patients.

Case example. Male, born 1961, was diagnosed with CML (classic Ph+ 100%) in 12/2007. Initial therapy, hydroxyurea 12/2007-03/2008. In 03/2008 imatinib 400mg/daily was started. No cytogenetic response was obtained up to 11/2010. Leukopenia (WBC 2.6x10^9/L) and thrombocytopenia (PLT 79x10^9/L) were revealed at check up before switching to second generation tyrosine kinase inhibitor therapy. Surprisingly, karyotype was as follows: 46,XY,(t;9;22)(q34;q11)(3)/45,XY,-7(9) with BCR-ABL level 3.887%. Trephine biopsy was performed and presented histologic signs of myelodysplasia. No BCR-ABL, NPM1, or FLT3 mutations were found. Status was evaluated as acquired myelodysplastic syndrome. Therapy was switched to dasatinib 100 mg/daily. At 3 months (46,XY,(t;9;22)(q34;q11)(11)/45,XY,-7(9)), BCR-ABL level 12.063%) and 6 months (46,XY,(t;9;22)(q34;q11)(3)/45,XY,-7(17), BCR-ABL level 7.555%) no significant effect of therapy was observed. On 10/2011 related a-lo-SCT with A locus mismatch was performed. Conditioning with fludarabine/busulfan/anti-thymocyte globulin was used. Engraftment (ANC>0.5x10^9/L, PLT>25x10^9/L) occurred at Day+11. Immuno-suppressive regimen with tacrolimus was used. Acute and chronic graft versus host disease appeared as skin impairment grade I-II. CCyR and MMR were reached at Day+35. At 6-month check-up there were stable CCyR and complete molecular response (4 log).

Conclusion
ACA in Ph- cells could be signs of BCR-ABL independent progression of oncogenic process. Prognostic role of ACA in Ph- cells needs to be assessed in large multicenter trials.
Objectives and background
Tyrosine kinase inhibitors (TKI) gave chance to increase life expectancy in CML patients. At the same time, continuous TKI treatment could bring various side effects that substantially affect quality of life. Grade 3/4 toxicities cause therapy interruption, which could decrease disease response. On TKI treatment grade 3/4 hematologic toxicity can occur in a considerable proportion of patients: anaemia in 5-20%, neutropenia in 12-58%, thrombocytopenia in 10-54% depending upon drug selection and line of treatment. In the early days of TKI implementation hematopoietic growth factors administration for hematological toxicity correction was overcautious in respect to the danger of disease progression. At present, most clinicians are not afraid to use erythropoiesis stimulating agents and granulocyte growth factor (G-CSF) for management of TKI-treatment side effects. Recently, immune thrombocytopenic purpura management has changed with introduction of thrombopoietin receptor agonists (TRA), which were highly effective and safe in such patients. It is possible that administration of TRA could significantly improve thrombocytopenic toxicity in CML patients.

We present a case report of correction thrombocytopenia with TRA in CML patient treated with Nilotinib.

Methods
Male, born 1960. CML with high risks on Sokal, Euro and Eutos scores was diagnosed in 05/2011. Imatinib 400 mg/daily was started as first-line therapy. Complete hematologic response was reached after one month of therapy. There were some side effects detected: edema grade I (successfully treated with diuretics) and chronic dermatitis grade II with no improvement on concomitant therapy. After 3 months of imatinib therapy there were no mytosis in cytogenetic and 21.432% BCR-ABL level. At 6 months (11/2011) there were no cytogenetic response (100% Ph+) with 49.249% BCR-ABL level. Patient was switched to nilotinib 800 mg/day on 29/11/2011. Nilotinib related side effects were mainly hematologic: neutropenia grade III and thrombocytopenia grade III 24/01-13/02/2012. This toxicity led temporarily to interruption of nilotinib and dose reduction to 400 mg/day. After 3 months of nilotinib there was no cytogenetic response (Ph+ 100%), but slightly decrease BCR-ABL level to 14.407%. Since 28/04/2012 neutropenia grade III and thrombocytopenia IV recurred. At check-up on 17/05/2012 there was minor cytogenetic response (Ph+ 55%). Unfortunately, thrombocytopenia and neutropenia were not improved until 17/05/2012. We tried to use G-CSF (filgrastim) 5 mcg/kg and TRA (romiplostim) 1 mg/kg to resolve hematologic toxicity.

Results
In control analysis (23/05/2012) one week after the first injection, neutropenia and thrombocytopenia were improved to grade II and III toxicity, respectively. The second administration of filgrastim and romiplostim led to complete neutrophil restoration and rising platelets to 61x10^9/l. Nilotinib administration was resolved with 400 mg/day dosage.

Conclusion
This case ground TRA potential to manage thrombocytopenia in CML patients on TKI treatment. Safety and efficacy of TRA administration in CML should be evaluated in further trials.

Figure 1. Neutrophils and platelet levels on G-CSF and TRA support.

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Introduction
In the last decade we have been witness to a great progress in molecular targeted therapy in many oncologic diseases, including chronic myeloid leukemia (CML). But it also opens the question of women in fertile age, who are planning pregnancy. Although tyrosine kinase inhibitors (TKI) have been in clinical practice more than 10 years, we know only a little about their impact on pregnancy. By now, published articles show that biological treatment as well as conventional chemotherapy has a potential negative effect on the foetus and so therapeutic management of these patients should start even in preconception period. According to actual knowledge, prescribing of TKI to pregnant women is not recommended because of their teratogenic effect.

Clinical Cases
1. 32 year old woman with CML started first line treatment with nilotinib 600mg daily in October 2008. She achieved complete molecular response (CMR) after 9 months. Her treatment was discontinued because of pregnancy after 18 months, so during the conception time she was still treated with nilotinib. The whole pregnancy she was without targeted therapy and she remained in complete hematologic remission (CHR) and major cytogentic remission (3% BCR-ABL-positive). Delivery was by caesarean section in October 2010, patient delivered a healthy boy with 3620g and 54cm. Because lactation was not recommended for her, she could start with nilotinib treatment immediately after delivery and she achieved CMR in 3 months.
2. 30 year old woman with CML is treated with imatinib 400mg daily since October 2008. After 18 months, this treatment failed and imatinib was changed to second generation of TKI - dasatinib 100mg daily. After 9 months she achieved CMR. After 16 months the treatment was discontinued because of her pregnancy, which means that during the conception time the patient was still treated with dasatinib. The whole pregnancy she was without targeted therapy and she remained in CMR and major cytogenetic remission (20% BCR-ABL-positive). Because of her hyper coagulation standing the whole gravidity was protected by LMWH. Delivery was natural in April 2012, patient delivered a healthy girl with 3450g and 50cm. Lactation was not recommended to her and she started dasatinib treatment immediately after delivery. After 3 months she achieved major molecular remission (MMR).

3. 31 year old man with CML is treated with imatinib 400mg daily since February 2008. After 18 months he achieved MMR. After 36 months the treatment was discontinued because of pregnancy planning. His female partner got pregnant in 2 months. The patient has started the imatinib treatment again without loss of optimal response and actually he is in CMR. His partner delivered a healthy boy with 3700g and 52cm in January 2012.

Conclusion
Treatment management in CML patients in fertile age, who are taking TKIs, begins in the preconception period with education about effective contraception. Women patients, who become pregnant during TKI treatment, have to consider the risk of development of foetal abnormalities by continuing treatment on the one side and the risk of losing optimal treatment response by treatment discontinuation on the other side. The lactation for patients using TKI is not recommended and they should resume treatment immediately after delivery.

[24] hOCT1 Gene Expression and BCR-ABL Transcript Level at 3 Months as Predictive Factors for Optimal Response to Imatinib Therapy in Patients with Chronic Myeloid Leukemia

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Objectives and background
Chronic myeloid leukemia (CML) is a myelo-proliferative neoplasm caused by formation of the BCR-ABL fusion gene and its product, BCR-ABL protein, showing a constitutive tyrosine kinase activity. The use of imatinib, the first tyrosine kinase inhibitor (TKI), leads to the achievement of durable hematologic, cytotgenic and molecular remission in more than 80% of patients. However, some patients do not respond to imatinib. In our previous study high expression of human organic cationic transporter-1 (hOCT1) which actively delivers imatinib into the cells was predictive for achievement of complete molecular response. The aim of this study was to evaluate the initial hOCT1 gene expression in correlation with BCR-ABL transcript level at three months as predictive factors for optimal response to imatinib therapy.

Methods
In 78 patients diagnosed with CML real-time quantitative polymerase chain reaction (RQ-PCR) assays were performed to investigate the expression of hOCT1 at diagnosis and BCR-ABL transcript level on the International Scale (BCR-ABL IS) after 3 months of imatinib treatment. At 18 months or earlier, response to therapy was evaluated according to the EAN criteria.

Results and statistical significance
The mean BCR-ABL transcript level at 3 months, in patients with high and low hOCT1 expression, was not significantly different. By 18 months, among patients with high expression of hOCT1, significantly more patients with a BCR-ABL transcript level at 3 months of 0.1-1% achieved optimal response than with a BCR-ABL transcript level of 1-10% (p=0.028). In a group of patients with a BCR-ABL transcript level at 3 months of 10% or more, high or low hOCT1 expression did not significantly influence the achievement of the optimal response. In both high and low hOCT1 expression groups, patients with a BCR-ABL transcript level at 3 months of 10% or more had their imatinib therapy changed to second line TKI significantly more often than patients with a BCR-ABL transcript level of 1-10% (p=0.028 p=0.015).

Conclusions
Low initial hOCT1 gene expression did not influence adversely on the chance to reduce the BCR-ABL transcript level at 3 months below 1% and to achieve an optimal response to imatinib. High (>10%) BCR-ABL transcript level at 3 months is an unfavourable predictive factor, and more significant than initial hOCT1 expression, for the optimal response to imatinib therapy and for the need for treatment change.
[25] Performance and Functional Status of Elderly Patients with Myeloid Neoplasms: How Does it Work?

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Objectives and Background
Traditionally, performance status (PS) is assessed by Karnofsky (KPS) and/or ECOG scales. Good PS is referred as KPS≥80% and ECOG≤2. It has been used as a criterion to include elderly in onco-hematologic protocols. Functional status (FS) is the patient’s level of independence in basic activities (such as food, personal hygiene, faecal and urinary continence) and instrumental activities of daily living, regarding more complex functions like phone use and locomotion capacity in the city. Geriatricians usually assess FS applying scales as basic and instrumental activities of daily living (ADL and IADL). The aim of this study was to evaluate the performance and functional status of elderly patients with myeloid neoplasms (MN) and to correlate these variables with age, sex and haemoglobin level.

Methods
The functional status of 67 elderly with myeloid malignancies, in the Hematology Out-patients clinics of UNIFESP, was assessed by KPS, ECOG, ADL and IADL. Good performance status was established when KPS ≥80% and ECOG=2. Patients without dependence in ADL and IADL were considered independent. Haemoglobin was measured within a maximum of 20 days before the functional status evaluation. A logistic regression model was applied to predict the influence of haemoglobin level, age and sex in functional status.

Results
GPS group had 53 patients and 49.1% were anaemic. 78.6% of fourteen patients with poor performance status had anaemia. On the other hand, only 28 patients were independent, 53.6% with anaemia. The area under the ROC curve (AUC) was 0.75, referring to model that included performance and haemoglobin. Considering dependence, the model with haemoglobin, sex and age had an AUC of 0.85.

Conclusions
This study showed the influence of sex, age and haemoglobin in performance and functional status which can be predicted by a logistic regression model. This approach aggregates helpful information on following up of elderly patients with NM: medical management may interfere in some variables, such as haemoglobin, in order to avoid deterioration in clinical condition and to preserve independence of elderly patients in daily activities.

[26] A Scientific Survey Studying the Belgian Myelofibrosis Patient Population According to Study Specific Disease Parameters

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Objectives and Background
The clinical presentation of myelofibrosis can encompass anaemia, splenomegaly, leukocytosis or leukaemia, thrombocytosis or thrombocytopenia and constitutional symptoms. In order to have a better insight of the distribution of distinct clinical features in the prevalent myelofibrosis patient population a scientific survey in 18 main haematology centres throughout Belgium was performed.

Methods
This scientific survey used a descriptive methodology to respond to a set of 76 pre-defined questions about prevalence and pre-defined study specific disease parameters. Data on prevalence and disease parameters were collected in each of the participating sites for all MF patients seen in clinic at each site in 2011. Only aggregated data from all sites were used for analysis. Data were collected for the most recent visit at the site, whether this was at time of diagnosis or when already under treatment. For patients currently being treated with ruxolitinib, data were from the last visit prior to starting this treatment.

Results
In this survey, 250 patients with myelofibrosis were captured; 165 (66%) of whom had primary myelofibrosis and 97 (39%) of whom were aged ≥65 years. Using these findings, combined with previously reported estimates of the prevalence of PMF (Rolison et al., 2008; Girordon et al., 2009) an estimated prevalence of 450 patients with primary or secondary myelofibrosis in Belgium was calculated, approximately 56% of whom were captured in this survey. Patients for whom data were reported in this survey are described according to parameters including IPSS and DIPSS risk category, spleen size and platelet count, as well as the presence of constitutional symptoms. The proportion of patients with a palpable spleen increases with the risk category (up to 81% in the IPSS high risk group and 95% in DIPSS high risk category with a spleen size ≥10 cm in 50% of these patients). In 32% of the low risk patients, the spleen was not palpable. Overall, 2% of the patients underwent splenectomy and 5% received earlier radiation of the spleen.

For myelofibrosis patients, the development of constitutional symptoms has an important impact on quality of life; about one third of all patients (34%) reported in this survey suffered from at least one of fever, night sweats and weight loss. The prevalence of constitutional symptoms also increased with the risk category. 80% of patients had a platelet count above 100.000/μl. Surprisingly, no association between platelet count and spleen size was found. Myeloblast count ≥1% occurred in 34% of patients and was associated with a larger spleen and presence of constitutional symptoms.

More results on the distribution of clinical features will be presented at the meeting.

Conclusions
The results of this survey provide some insight into the characteristics of the Belgian myelofibrosis population, according to specific disease parameters.
[27] Predictive Relevance in Leukemic Progression of CBL, DNMT3A, TP53 and IDH1/2 Mutation in Patients with Primary Myelofibrosis

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Background
Among MPN cases, those presenting with myelofibrosis (MF) have the worst prognosis. Previous prognostic studies which were focused on risk factors for overall survival resulted in the establishment of several prognostic scoring systems; however, to identify pts with very high risk disease would be particularly useful for therapeutic choices included HSCT. Recent studies have reported higher frequencies of IDH1/IDH2, DNMT3A, CBL and TP53 mutations in blast-phase MPN, suggesting a contribution to disease progression.

Methods
We genotyped by direct sequencing at diagnosis 520 pts with PMF for mutations of CBL (exon 8, 9), DNMT3A (exon 5-23), IDH1 or IDH2 (exon2 or 4, respectively). We also evaluated mutational status of TP53 (exon 1-10) at diagnosis in only patients that progressed to leukemia.

Results
Median pts FU was 44mo (2-340); 164pts died (31.8%), 81 (18.3%) because of leukemia (AL); the global LFS was 21.2 yrs. PMF pts survival differed significantly according to the IPSS and DIPSS category. CBL mutation. We found 20 (4.3%) mutated pts, harbouring 20 different exonic mutations. Leukemia occurred in 28.5% of CBL mutated and 15.4% of wt pts; however leukemia free survival (LFS) was not statistically significant (P=.07). DNMT3A mutation. Among 490 evaluated patients we found 19 mutations in 28pts (5.7%); leukemia occurred in 14.3% of DNMT3A mutated and 15.8% of wt pts (P=ns) with a LFS not statistically different (P=.566). IDH1/2 mutation. We found 11 (2.2%) patients mutated, 5 in IDH1 (1%) and 6 (1.2%) in IDH2. Progression to leukemia was observed in 54.5% of IDH mutated and 14.7% of wt pts. According to the mutational status LFS was significantly shorter in mutated compared to wt pts (254 vs 46.2mo, respectively, P<0.0001). No impact of CBL, DNMT3A and IDH1/2 mutations on OS were seen. Among 44 pts who progressed to leukemia only four (9%) presented TP53 mutation at diagnosis.

Conclusion
Overall, these results showed that mutations in genes previously associated with blast phase of MPN such as CBL, DNMT3A and TP53 did not allow prediction of a greater risk of leukemic transformation when assayed at the time of diagnosis; on the other hand, IDH1 or IDH2 mutations correlated with a shorter LFS, supporting a rational for their inclusion as predicted factors of leukemic transformation already at the time of diagnosis.

[28] Genetic Abnormalities in Diagnostics of BCR-ABL-Negative Myeloproliferative Neoplasms

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Objectives and background
Genetic mutations result in abnormalities of myelopoietic proteins and lies at the base of myeloproliferative neoplasms (MPNs) development and subsequent progression. The aim of our study was to assess frequencies of JAK2 MPL mutations and cytogenetic aberrations in patients with BCR-ABL-negative MPNs.

Methods
Blood samples from 567 patients with BCR-ABL negative MPNs were selected. There were 224 cases of polycythaemia vera (PV), 102 cases of essential thrombocythaemia (ET), 102 cases of primary myelofibrosis (PMF) and 140 cases of chronic myeloproliferative disease, unclassified (CMPD-U).

Results
Frequency of the JAK2 V617F allele was 3.9% (5 cases) karyotypes with isolated chromosomal aberrations (del(20q), del(13q) (favourable prognosis), 6.2% (8 cases) karyotypes (interim risk), and 4.7% (6 cases) karyotypes with integrated violations (unfavourable prognosis). Chromosomal aberrations which cause favourable prognosis were defined reliably more often in PV cases than in PMF cases (p<0.00001). Moreover, the frequency of karyotypes with integrated violations was statistically higher in PMF cases as compared with PV and ET cases (p<0.00001). Two of 6 patients with integrated violations in karyotypes had transformation from MPN to AML.

Conclusion
Point mutations in JAK2 and MPL genes are specific markers for patients with BCR-ABL-negative MPNs. Integration molecular genetics with cytogenetics helps to stratify patients into different risk groups and optimize the treatment strategy.
[29] LNK Mutations in JAK2 and MPL Negative Essential Thrombocythemia Patients

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Abstract

Chronic myeloid leukemia (CML) was described in the 19th century as a primary distinct disease entity. The presence of the minute Philadelphia chromosome (Ph1) is part of the (t9;22)(q34;q11) and diagnostic for CML. Three Dutch investigators unravelled the molecular etiology of Ph1-positive CML. C-ABL moved from 9q to 22q. The Ph1 chromosome breakpoints on chromosome 22 in CML patients are clustered within a limited region, BCR-ABL. It became evident that the BCR-ABL fusion gene resulted in high tyrosine kinase activity. The BCR-ABL fusion gene is found in classical CML patients with the (t9;22)(q34;q11) translocation but also in CML patients with complex chromosomal translocations. Ninety percent of patients with the clinical phenotype of CML are Ph1+. The latter group are usually diagnosed as atypical CML, juvenile CML, chronic neutrophilic leukemia or chronic myelomonocytic leukemia. The diagnosis of CML is made by its conspicuous increase of metaplasia of the spleen and myelofibrosis when compared to BRC-ABL-Negative Essential Thrombocythemia and Chronic Myeloid Leukemia: Diagnostic Differentiation at the Clinical and Bone Marrow Pathology Level

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Abstract

Chronic myeloid leukemia (CML) was described in the 19th century as a primary distinct disease entity. The presence of the minute Philadelphia chromosome (Ph1) is part of the (t9;22)(q34;q11) and diagnostic for CML. Three Dutch investigators unravelled the molecular etiology of Ph1-positive CML. The Ph1 chromosome breakpoints on chromosome 22 in CML patients are clustered within a limited region, BCR-ABL. It became evident that the BCR-ABL fusion gene resulted in high tyrosine kinase activity. The BCR-ABL fusion gene is found in classical CML patients with the (t9;22)(q34;q11) translocation but also in CML patients with complex chromosomal translocations. Ninety percent of patients with the clinical phenotype of CML are Ph1+. The latter group are usually diagnosed as atypical CML, juvenile CML, chronic neutrophilic leukemia or chronic myelomonocytic leukemia. The diagnosis of CML is made by its conspicuous increase of granulopoiesis in the bone marrow with enlarged zones of proliferating blasts and promyelocytes, grouped in seams along the bone trabeculae and characteristic small megakaryocytes with round nuclei, showing only little lobulation and could be termed as CML-megakaryocytes. Three phenotypes of BCR-ABL-positive MPN can be distinguished; CML with megakaryocyte predominance (CML-M) and CML of common type (CML-CT) and BCR-ABL+ ET. Life expectancy did not significantly differ among the subtypes of CML-CT and CML-M, but patients with CML-M have a higher risk of developing myelofibrosis (MF). According to strict morphological, biochemical, and molecular criteria, BCR-ABL-positive CML is a malignant disease (real neoplasia) with an obligate transition into acute leukemia. Patients with benign myeloproliferative disease (essential thrombocythemia, polycythemia vera) and primary megakaryocytic myeloproliferation (PMGM), show a low tendency to leukemic transformation (neoplasia) but myeloid metaplasia of the spleen and myelofibrosis predominates. The increase of small monocellular megakaryocytes in bone marrow smears and biopsy is a clue to the diagnosis of Ph1+ essential thrombocythemia (ET) and part of the BCR-ABL+ CML neoplastic malignancy. Life expectancy may progress to CML and show a high tendency to myelofibrosis. Both BCR-ABL+ ET and BCR-ABL+ thrombocythemia associated with CML can be regarded as early manifestations of the chronic stable phase of CML. Despite a high platelet count of between 400 and 1000 x 10^9/L, and even when in excess of 1500 x 10^9/L, patients with BCR-ABL+ ET did not present with the erythromelalgic thrombotic or bleeding manifestations seen in JAK2V617F mutated thrombocythemia in various MDS stages. BCR-ABL+thrombocythemia, small, indolent and non reactive platelets originate from small megakaryocytes with hypolobulated nuclei and do not cause platelet mediated erythromelalgic microvascular circulation disturbance.
[31] Fulminant Course of FIP1L1–PDGFRA Positive Myeloproliferative Disorder with Central Nervous System Involvement

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Background
FIP1L1–PDGFRA rearrangement is a common cause of chronic myeloproliferative disorder (MPD) with eosinophilia. It is characterized by both the typical features of myeloproliferation such as leucocytosis with left shift, splenomegaly, bone marrow hypercellularity with eosinophils and neutrophils enlargement and symptoms due to hypereosinophilia (most often organ damage). Imatinib in a low dose is an effective target-directed therapy in PDGFRα positive MPD. Central nervous system (CNS) involvement is a second severe complication of the hypereosinophilia and a cause of death in some cases. But, in PDGFRα positive MPDs, CNS involvement is quite a rare event. We know only two reports in the literature describing this complication in PDGFRα positive MPD (N Frichhofen, Ann Hematol 2004, C Williams, J Clin Pathol 2008).

Aim
We report a case of this disease with fulminant course and severe CNS damage. Case report: A 39 year-old woman was admitted to our observation with fatigue and fever. Leukocytes, platelets and hemoglobin were reduced in the peripheral blood whereas a hypercellularity with the prevalence of immature eosinophils were found in the bone marrow. After a short period of pancytopenia eosinophilia rapidly increased (from 700/μl/mm to 86000/μl/mm) and was accompanied by the appearance of ataxia, amnesia, mental impairment and tetraparesis over 6 days. Neurological symptoms were strongly correlated with the increase of eosinophilia. Magnetic resonance imaging (MRI) indicated a multifocal damage of the white substance of brain (hemispheres, cerebellum). Total infiltration of lung and breath deficiency was the reason for starting artificial lung ventilation (ALV). Therapy with imatinib at 400mg o.d. was started and resulted in normalization of leucocyte eosinophil count after 3 weeks and PCR negativity after 4 weeks. Because of ALV imatinib was given through the nasogastric tube during first 3 weeks. Neurological symptoms subsequently improved at the end of second month imatinib therapy. The mental functions were restored and tetraparesis partly resolved.

Conclusion
There are a few mechanisms of CNS damage in a hypereosinophilic syndrome. It is known that some eosinophilic proteins have a neurotoxic property leading to demyelination. And other causes are thromboembolism of the brain vessels and stasis due to hyperleucocytosis. Marked eosinophilia allows suspect both neurotoxic effect and vessels occlusion in this case. Targeted therapy with imatinib saved the life of our patient but a higher dose than in most cases was required.

[32] mRNA Expression Levels of Local Renin-Angiotensin System in Lymphoid and Myeloid Hematological Malignancies

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Objectives and Background
The renin-angiotensin system (RAS) has been considered as an endocrine system that controls the blood pressure and fluid-electrolyte balance. RAS is also present in specific tissue systems locally having autocrine/paracrine and even intracrine functions. The local bone marrow renin-angiotensin system has also been defined as an autocrine-paracrine system affecting normal and neoplastic hematopoiesis. Angiotensin II, which is produced by the angiotensin converting enzyme (ACE), stimulates proliferation and differentiation of hematopoietic stem cells through Angiotensin II type 1 receptors.

Methods
In this study, peripheral blood samples of 46 patients (mean age: 57.8±13.5) were collected and classified as lymphoid (n=30) group and myeloid (n=16) during their routine investment and diagnosis at Hacettepe University Faculty of Medicine, Hematology Unit. Renin, ACE, ACE2 and ANGTS mRNAs levels have been investigated using related primers by using quantitative real time polymerase chain reaction.

Results and significance
In the lymphoid group, the median expression values of renin, ACE, ACE2, and ANGTS mRNAs were 1.96%, 0.42%, 0.00%, and 0.00%, respectively; in myeloid group 0.73%, 1.55%, 0.04% and 0.01%, respectively. In the lymphoid group, renin levels were significantly higher (p=0.001), whereas ACE1 and ACE2 levels were significantly higher in the myeloid group (p values were 0.013 vs. 0.010, respectively). ANGTS levels were similar in both groups. The all patients with active disease have had significantly higher renin mRNA expression levels, than those without an active disease (2.0316 vs 0.2969) (p=0.034).

Conclusions
Renin levels were significantly higher in the lymphoid group, whereas ACE levels were significantly higher in the myeloid group. Moreover, renin levels in all patients with active disease were significantly higher than those without an active disease. First evidences of possible effects of the local bone marrow RAS components on lymphoid and myeloid malignancies have been demonstrated in this study.

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Background
Despite the advent of new drugs allo-HSCT remains the only effective treatment of high risk AML. The aim of the study was to estimate long term OS (5 years), to find out the main factors which predict the AML treatment results after allo-HSCT.

Patients and methods
We analyzed the results of 104 AML patients (pts) after allo-HSCT (MFD – 41, MUD – 63). The median age was 29 (15-68) years. Ninety one (88%) cases of AML were de novo, 13 (12%) have secondary AML. Thirty three patients (32%) were in CR1, 31 (30%) was in CR2/CR3 and 33 (32%) patients was not in remission at the moment of transplantation. Cytogenetic analysis at diagnosis was performed in 71 case, 10 (10%) patients was of favourable risk, 47 (45%) of intermediate and 14 (13%) of unfavourable risk. Myeloblastic conditioning (MAC) was admitted in 33 (32%) pts, reduce intensity conditioning in 33 (32%) pts, reduce intensity conditioning and 14 (13%) of unfavourable risk. Myeloblastic conditioning (MAC) was admitted in 33 (32%) pts, reduce intensity conditioning and 14 (13%) of unfavourable risk.

Introduction and Background
Less than 20% of elderly patients are cured of acute myeloid leukemia with intensive chemotherapy. We analyze the efficacy and overall safety of 5-Azacitidine in elderly AML patients with severe co-morbidities, correlated with immuno phenotype data.

Methods: From September 2009 to April 2012, 10 elderly patients (8 de novo and 2 secondary AML with median age 73 years, range 65–81 years) diagnosed with non-promyelocytic AML (not eligible for standard induction chemotherapy), signed informed consents and received Azacitidine (75 mg/m²/d) for 7 days of every 28-day cycle until loss of response or disease progression in our institution. The patients presented severe co-morbidities (hepatopathy HCV correlated, ischemic cardiopathy, chronic renal failure, BPCO, diabetes). Cytogenetic analysis was performed and showed normal karyotype in 6 patients, in 3 patients monosomy of chromosome 5 and 7, and in 1 patient chromosome 5q deletion. Immunophenotypic analysis of bone marrow samples was evaluated at diagnosis and during follow up (each six months) in 10 AML patients. Positivity was defined as more than 20% blasts expressing a specific antigen. It was possible to evaluate the percentage of positive blasts for the following antigens: CD33, CD13, HLA-DR, CD14, CD34, CD11b, and CD117.

Results and Statistical Significance
Median white blood cells was 4.4 x10³ µl, range(1.2-16 x10³ µl) Of the patients that during treatment down expressed positivity of surface antigen blasts <20% showed response to therapy. At onset patients with acute myeloid leukemia had a medullary blast count of 20–50%. Patients received a median number of 14 cycles of therapy (range 7–24). For hematological improvement (HI) we considered stable disease but reduced need to supportive care(red cell and platelet transfusions ).We evaluated overall response to treatment in 80%(CR5pts + PR1pt + NR but reducted need to supportive care)regimen and transplant source have no correlation, ischemic cardiopathy, chronic renal failure, BPCO, diabetes). Cytogenetic analysis was performed and showed normal karyotype in 6 patients, in 3 patients monosomy of chromosome 5 and 7, and in 1 patient chromosome 5q deletion. Immunophenotypic analysis of bone marrow samples was evaluated at diagnosis and during follow up (each six months) in 10 AML patients. Positivity was defined as more than 20% blasts expressing a specific antigen. It was possible to evaluate the percentage of positive blasts for the following antigens: CD33, CD13, HLA-DR, CD14, CD34, CD11b, and CD117.

[33] Allogeneic Hematopoietic Stem Cell Transplantation in Patients with Acute Myeloid Leukemia: Single Centre Experience


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Results
The incidence of acute GVHD grade 3-4 after allo HSCT from MFD was 20%, after allo HSCT from MUD – 35% (p=0.121), the cumulative incidence of extensive chronic GVHD was 9.8% and 38.4% respectively (p=0.001). OS at 5 years after allo HSCT from MFD in CR1 was 51%, in CR2/CR3 – 28%, not in remission – 14% (p=0.0008); after allo HSCT from MUD – 60%, 23% and 7% respectively (p=0.0093). The main cause of death after allo HSCT from MFD was relapse (67%). In pts who were transplanted in CR1 OS at 5 years after MAC allo HSCT was 67%, after RIC allo HSCT – 53% (p=0.6788); transplanted with bone marrow 64 %, with peripheral stem cells – 53% (p=0.3766).

Conclusion
The incidence of acute GVHD grade 3-4 after allo HSCT from MUD was similar – 20% vs 35%. The cumulative incidence of extensive chronic GVHD was significant higher in pts after allo HSCT from MUD – 9.8% vs 38.4%. Long term OS at 5 years after allo HSCT from related and unrelated donor was comparable. Status of the disease at the moment of transplant was the main factor which significantly influenced to long term outcomes in AML pts after allo HSCT. In this study the best outcome was achieved in pts who were transplanted in CR1 (OS at 5 years was more than 50%). The conditioning regimen and transplant source have no significant influence to long term OS in this group (CR1).

[34] Monitoring Response to Treatment and Minimal Residual Disease (MRD) by Flow Cytometry in Elderly Patients with Acute Myeloid Leukemia Treated with 5-Azacitidine: A Single Centre Experience


Introduction and Background
Monitoring response to treatment and minimal residual disease (MRD) is a critical tool in the management of acute myeloid leukemia (AML). Multi-parametric flow cytometry (MPFC) is an alternative method to quantify MRD. The flow cytometric abnormalities that characterize the leukemia blasts were reduced in PR patients and absent in CR patients. Besides using flow cytometry as monitoring method of MDR we noticed that in patients would lose the response to therapy after few months showed an increased flow cytometry positivity that predicted clinical outcome of patients (P<0.01). All patients with normal karyotype (p=0.001) reached complete and lasting remission.

Conclusions
In our limited experience in these subgroups of elderly patients with AML, 5-azacitidine prolongs survival and is well tolerated even in patients with a higher degree of bone marrow blasts (>30%) and with severe co-morbidities in a subset of patients that could not tolerate AML like therapy but only supportive care.
[35] The Evaluation of Hematopoietic Niche Proteins in Patients with Acute Myeloid Leukemia

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Objectives and background
Angiogenesis plays a pivotal role in AML development. The microenvironment of the hematopoietic niche is considered crucial for leukemic stem cells’ self-renewal and quiescence. Previously we have found that the number of circulating endothelial cells (CEC) in the peripheral blood of AML patients is a non-invasive marker of angiogenesis. Osteopontin (OPN), angiopoietin-1 (ANG-1), Notch-1 receptor and its ligand, Jagged-1, are the main components of the hematopoietic niche, and this as well as their angiogenic potential, have been reported. This study was conducted to elucidate the clinical role of the hematopoietic niche proteins in AML patients. The level of circulating OPN, ANG-1 and the expression of Notch-1 and Jagged-1 on leukemic blasts were assessed. Additionally, the level and expression of examined proteins were correlated with known prognostic factors and CEC number.

Methods
OPN and ANG-1 level were measured in plasma samples of 137 newly diagnosed AML patients with a median age of 59 (range 18-78 years) and 16 controls using ELISA method. Notch-1 and Jagged-1 expression were assessed on leukemic blasts of 75 AML patients with a median age of 58 years (21-82y). The percentage of Notch-1 and Jagged-1 positive cells served as protein expression. The median (Me) level of proteins was used to subdivide patients into “low-expressers” and “high-expressers” groups. Numbers of CEC sub populations as well as Notch-1 and Jagged-1 expression were evaluated by multicolour flow cytometry.

Results and statistical significance
In AML patients the median OPN level was significantly higher (157.5 pg/mL) whereas the level of ANG-1 (Me 894 pg/mL) was lower than in the control group (Me 70 pg/mL; p=0.00001; 1710 pg/mL; p=0.003, respectively). The median expression of Notch-1 and Jagged-1 were 2.2% (range 0.1-24.8%) and 20.6% (range 0.9-89.7%); respectively. We observed a significant correlation between Jagged-1 and Notch-1 protein expression on AML blasts (r=0.24; p=0.048). The lower expression of Notch-1 (<Me) was associated with a higher number of CECs derived from the microvascular network (CEC CD36+: p=0.04). Moreover there were trends to negative correlation between Notch-1 expression and total CEC number (p=0.06), and activated CEC (aCEC; p=0.06) and circulating endothelial progenitors (CEP; p=0.06). Interestingly, lower expression of Notch-1 receptor correlates strongly with good cytogenetic risk according to SWOG classification (p=0.02). We observed lower expression of Jagged-1 in patients with peripheral blast counts >Me (p=0.02) and WBC >20 G/L (p=0.06). The significantly higher level of plasma OPN was observed in patients with a haemoglobin level <10 g/dL (p=0.008) and elevated LDH activity (p=0.05).

ANG-1 level was higher in patients with bone marrow infiltration >60% (p=0.05) and WBC >20 G/L (p=0.03) and lower percentage of CEC CD36+ (p=0.05).

Conclusions
Our observations indicate that hematopoietic niche proteins play an important role in AML pathogenesis. Jagged-1 is highly expressed on AML blasts and correlates with lower tumour burden in AML patients. The reverse correlation of Notch-1 expression and ANG-1 level with number of CECs and their sub populations may suggest a negative impact on angiogenesis in AML. A better understanding of the precise functions of hematopoietic niche proteins may create new options for therapeutic interventions in AML.

[36] Profiling and Targeting Leukemic Stem Cell Specific microRNAs in AML

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Background
Despite high remission rates after chemotherapy, only 30-40% of acute myeloid leukemia (AML) patients (<60 years) survive five years after diagnosis. For elderly patients (>60 years) the survival rate is only 10-20%. The main cause of present treatment failure is thought to be the insufficient eradication of leukemic stem cells (LSCs) causing relapse, with, moreover, non-specific elimination of hematopoietic stem cells (HSCs) resulting in toxicity. The eradication of LSCs is therefore necessary to cure AML patients and success of anti-LSC therapy will rely on eradication of LSCs while sparing HSCs. For the development of LSC-specific therapies more insight in the molecular and functional differences of HSCs, LSCs and malignant progenitors is needed.

miRNAs (miRNA) are small non-coding RNAs which regulate gene expression by targeting mRNAs. Since miRNAs are involved in processes like proliferation, differentiation and apoptosis and possibly affect many pathways simultaneously they could be promising therapeutic targets. We have identified miRNAs that are differentially expressed between HSCs, LSCs and the malignant progenitors all isolated from single AML bone marrow samples. Moreover, we showed that targeting of several of these identified miRNAs induced apoptosis.

Methods
We have recently identified aldehyde dehydrogenase (ALDH) activity as a functional discriminative difference between HSCs and LSCs within the CD34+CD38− compartment of the AML bone marrow. HSCs show high ALDH activity whereas LSCs have lower activity. In this study, we have used ALDH activity for identification and purification of HSCs, LSCs and leukemic progenitors all derived from the same AML bone marrow (n=6). miRNA expression within these different cell fractions was investigated by using microarray analysis and expression profiles were validated using qRT-PCR. Next, we modulated the expression of the identified miRNAs in AML cell lines and primary AML samples using lentivirus and studied the effects on proliferation, apoptosis, chemotherapy sensitivity and colony forming capacity.

Results
Microarray analyses revealed differential expression between LSCs and HSCs of miR-21, miR-181a/b, miR-551b, miR-29b and miR-125b. MiRNAs with differential expression between LSCs and leukemic progenitors were miR-126/miR-126*, miR-335, miR-146a and miR-1260. We confirmed the expression patterns of several of the identified miRNAs by qRT-PCR. The down regulation of some of these miRNAs induced apoptosis in AML cell lines as well as in primary AML cells and reduced colony formation in a CFU-assay. Furthermore, inhibition of these miRNAs reduced tumor growth in an AML xenograft mouse model.

Conclusion
In conclusion, we have identified several miRNAs that are differentially expressed between LSCs and HSCs and LSCs and leukemic progenitors. These miRNAs might characterize and potentially maintain AML LSCs through their effects on proliferation, apoptosis and differentiation. Targeting of some of these miRNAs can induce apoptosis and may in the future be used as valuable anti-leukemic miRNA-based therapeutics.
[37] Biology and Therapy of Leukemias and Myelodysplastic Syndromes (MDS) with Higher EVI-1 Gene Expression

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Background
Recent investigations have shown, that non-random EVI-1 gene over expression may be present in patients (pts) with chemotherapy resistant acute myeloid leukemias (AML) and MDS [Lugthart et al., 2011], as well as in pts with advanced stages of chronic myeloid leukemia (CML) who do not respond to tyrosine kinase inhibitors (TKI) without an absence of T315I BCR-ABL gene mutations [Daghistanti et al., 2010]. Moreover, the allelicne hematopoietic stem cell transplantation (HSCT) provided good 5-year survival in the CML pts. The aim of the study was to assess the frequency of EVI-1 positive AML and MDS in pts with different cytogenetic sub-variants and to evaluate some peculiarities of clinical course, including their responses to chemotherapy and HSCT.

Material and methods
Standard cytogenetics supplemented by multicolour FISH and molecular testing of EVI-1 gene expression after Gröschel et al. [2010] were performed in 230 pts, being over expressed in 27 of them. The mean age of pts at diagnosis was 32.7 years (range from 2 to 80 years). Among them were pts with AML, MDS, CML and Ph+ ALL (12, 3, 14, and 1 pts, respectively).

Results
The involvement of 3q26 locus, which has been recently considered as a main reason for EVI-1 over expression, was revealed by us in 4 pts, including 1 pts each with AML, EMS (8p11) syndrome, MDS and BC of CML. Monosomy 7 was associated with EVI-1 over expression in 4 pts whereas arrangements of MLL gene took place in 2 others. In addition, the higher EVI-1 gene expression was noticed in 14 pts with CML and 1 pt with Ph+ ALL who were resistant to the therapy by TKI. Moreover, EVI-1 over expression was noticed here in 2 pts with t(8;21) (at the first time), complex (n=3) and normal (n=2) karyotypes. HSCT was carried out in 10 pts. It was allo MFD (n=1) or MUD (n=8), auto (n=1) and haplo transplantsations. Six of 10 pts (60%) on the day of HSCT were at relapses, 3 at the 1st and one at the 3rd remissions. As a result, 4 of 10 operated pts are alive, including all with first remissions. Death was registered 3 to15 months after HSCT. Among death reasons were leukemia relapses, graft failure and infective complications (4, 1, and 1 cases, respectively).

Conclusion
EVI-1 over expression occurs in 10% of pts with different cytogenetic sub-variants of leukemias and MDS. Since these leukemias are prognostically pure, their diagnosis followed by alloHSCT should be done as soon as possible. The main candidates for molecular study must be AML and MDS pts with monosomy 7, 3q26 and 11q23 loci rearrangements, and those with CML who reveal a resistance to TKI therapy with no T315I mutation of gene BCR-ABL.

[38] Is Standard Treatment (DA 3i+7) Still Recommended as Front Line Therapy for Adult Patients With De Novo AML? The Role of Etoposide

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Background
Early intensification of chemotherapy in the remission induction phase of patients with acute myeloid leukemia (AML) has been shown to improve both the remission rate and long-term leukemia-free survival.

Aim
To assess the role of etoposide in the induction remission schedules in younger patients with de novo AML.

Methods: Newly diagnosed previously untreated younger patients (15-60 years) with AML were assigned into 3 groups: A, B, and C. Group A received conventional treatment DA 3i+7 [daunorubicin 60 mg/m2/day (days 1-3) and Ara-C 200 mg/m2/day] by continuous IV infusion (days 1-7). Group B received [Ara-C 100 mg/m2/day] by continuous IV infusion (days 1-10) + daunorubicin 50 mg/m2/day (days 1, 3, 5) + etoposide (50 mg/m2/day; days 1-5). And group C received high dose AraC (HD-AraC) 3g/m2/12 hrs. (days 1, 3, 5 and 7) + daunorubicin and etoposide in the same dose as above. All patients were treated in the Department of Hematology, University Hospital Bratislava. Informed consents were obtained. RT-PCR was done to determine the expression of topoisomerase I, II-alpha and II-beta genes in bone marrow samples at diagnosis. The data were analyzed using SPSS statistical software versions 16.0, 2008.

Results
Between September 2000 and August 2011, 128 patients were recruited (group A = 27, group B = 57, and group C = 44). There was no statistically significant difference in age, sex, performance state, AML subtype, WBC count and cytogenetic risk distribution between the 3 groups. Patients were followed for a median of 92 months. After 1 course of induction the complete remission (CR) rates were 55.6%, 75.4% and 81.8% for groups A, B and C respectively (P = 0.048). Group A vs. B (P = 0.025), group A vs. C (P = 0.027), group B vs. C (P = 0.81). Induction toxicity profile and grade were similar in all groups except for conjunctivitis grades 2 and 3 which were higher in group C. The 5 year overall survival (OS) was 17%, 41% and 35% (P = 0.36), and disease free survival (DFS) was 25%, 44% and 35% (P = 0.21) in groups A, B and C respectively. There was a statistically significant improvement in OS but not DFS in patients receiving etoposide (P < 0.00001). Over expression was detected in 12/19 (63%) and 14/19 (74%) patients for topoisomerase I and II genes respective- ly. Topoisomerase II-alpha and II-beta genes were increased by a median of 4.1 (range 1.7-27.3) and 2.9 (range 2.4-5.1) than normal, respectively.

Conclusion
The addition of etoposide to the standard treatment of AML can improve the rate of complete remission and outcome in adult de novo AML patients. The incorporation of etoposide to HD-AraC regimens during induction may further improve the rate of CR without an effect on outcome (DFS and OS). Measurement of topoisomerase II gene expression at diagnosis can help in selecting patients who will benefit from the addition of etoposide.
Background
Acute myeloid leukemia (AML) represents a heterogeneous disorder with recurrent chromosomal alterations and molecular abnormalities. Among AML with normal karyotype (NK AML) FLT3 activating mutation, internal tandem duplication (FLT3-ITD), is present in about 30% of patients, conferring unfavourable outcome. Our previous data demonstrated specific up-regulation of miR-155 in FLT3-ITD+ AML. miR-155 is known to be directly implicated in myeloid hyperplasia and/or hematopoiesis. Our aim was to integrate data from different sources such as the GEO gene expression database and MRD@NT@N prediction tool to model the role of miR-155 in FLT3-ITD+ AML and to validate this by experimental analysis.

Method
In this study we applied a four step strategy. At the first step, using gene expression dataset from GEO database, we generated the transcription factors co-regulation network acting in FLT3 mutated AML and at the same time, we predicted the miR-155-TF connections by MRD@NT@N. In the second step, we extracted, from the general network, the module of transcription factors connected to miR-155. At the third step, we compared the miR-155 module with the canonical pathways. At the final step, using a new cohort of newly diagnosed AML patients, we verified the expression levels of most intriguing hubs and correlated them to miR 155 expression levels. From these analyses, we derived a sub-network, called “miR 155 module” that describes functional relationship among miR 155 and transcription factor in FLT3-ITD+ AML.

Results
We confirmed a strong up regulation of miR 155 in the FLT3 ITD+ AML. We found that “miR 155 module” is characterized by the presence of six transcription factors as central hubs: four miR 155 regulators (JUN, RUNX1, FO5b, JUNB) and two targets of miR 155 (PU.1, CEBP) all known to be “master” genes of myelopoiesis. We found, in FLT3 ITD+ AML, a significant down regulation of miR 155 target genes CEPI beta and PU 1 (respectively 0.354 fold p=0.000 and 0.404 fold p= 0.000) and up regulation of miR 155 regulator genes JUN and RUNX1 (2.597 fold p=0.0210 and 2.640 fold p=0.0001 respectively). We described, for the first time, a regulatory pathway that connects FLT3 ITD mutation, a poor prognostic marker for AML, to reduced expression of TFs master regulators of myelopoiesis. Our results suggest that activating mutations of FLT3 in AML can, through the induction of JUN, lead to increased expression of miR 155, which then causes down regulation of PU.1 and CEBP beta and consequently causes a block of myeloid differentiation. More simply, FLT3-ITD → JUN → miR-155 → iPU.1 → iCEBPbeta → iMyelopoiesis.

Conclusion
Our study consolidates data on miR 155 association with FLT3 ITD+ AML, describes an integration of sequence based prediction analysis with expression network that individuates vertices involved in the molecular pathogenesis of FLT3 mutated AML, suggests a molecular pathway that starting from in FLT3 activating mutation, through miR-155, damages myeloid differentiation. We also suggest that miR 155 deregulation may act as a central hub in the multistep mechanism of FLT3 mutated leukemogenesis therefore offering new therapeutic strategies. This work was supported by a grant from Associazione Italiana Ricerca sul Cancro (Project IG 10701 AIRC).

[39] miR-155 Regulative Network in FLT3 Mutated Acute Myeloid Leukemia

[40] Azacytidine Impairs NK-Cell Activity in AML and MDS Patients Undergoing MRD-Based Pre-emptive Treatment after Allogeneic Stem Cell Transplantation
A monosomal karyotype (MK) defined with banding techniques (BT) by Breems et al. is associated with a particularly poor prognosis in acute myeloid leukemia (AML), and overall survival (OS) of patients with complex karyotype (CK) and MK is even worse than for CK(+)MK(-) patients. However, BT may be insufficient to determine the actual loss of a complete chromosome, especially in patients with CK where parts of “missing” chromosomes could be involved in various structural aberrations. Molecular cytogenetic techniques, such as fluorescence in situ hybridization (FISH), could be useful to verify whether observed monosomy is total (loss of a complete chromosome) or partial (loss of only a part of a chromosome, including centromere). The aim of our study was to assess if the type of monosomy (total or partial), defined using FISH influences the prognosis of CK-AML patients.

Methods
The study covered 80 newly-diagnosed CK-AML patients [≥3 aberrations, not including t(8;21)/inv(16)/t(15;17)] treated between 2005-2012 with PALG AML1/2004 and AML2/2004 protocols. Conventional cytogenetics was performed at diagnosis using standard BT. Karyotypes were reported in accordance with ISCN 2009. In all patients with MK determined by BT, FISH with whole-chromosome painting probes was performed. Patients were divided into three groups: 1)CK(+)MK-t: CK with the presence of at least two total monosomies confirmed by FISH or at least one total monosomy confirmed by FISH, accompanied by structural aberrations, irrespective of number of partial monosomies, 2)CK(+)MK-p: CK without any total monosomy confirmed by FISH and with at least one partial monosomy, and 3)CK(+)MK(-): CK without any type of monosomy.

Results and significance
The median age of 80 CK-AML patients was 59 years (range 19-79 years). Forty one (51%) patients received intensive induction chemotherapy according to PALG protocols, 19 patients - low dose cytarabine and 20 patients with high frailty index received best supportive care. In 30/80 (37.5%) analyzed cases a total monosomy was confirmed by FISH or at least one total monosomy confirmed by FISH, accompanied by structural aberrations, irrespective of number of partial monosomies, 2)CK(+)MK-p: CK without any total monosomy confirmed by FISH and with at least one partial monosomy, and 3)CK(+)MK(-): CK without any type of monosomy.

Conclusions
Our results indicate for the first time that only total but not partial monosomy is associated with significantly shorter OS compared to CK(+)MK(-) patients. These data clearly demonstrate the important role played by FISH in the precise evaluation of complex karyotypes in routine diagnosis, which facilitates both correct classification within the MK group and an adequate description of monosomy type.
Myelodysplastic syndromes (MDS) comprise a heterogeneous group of hematopoietic stem cell disorders, characterised by ineffective hematopoiesis resulting in cytopenias and a variable risk of acute myeloid leukemia. The finding of hematopoietic cells with an abnormal immunophenotype by flow cytometry (FC) in bone marrow (BM) of patients with MDS is of prognostic relevance and can aid in treatment decision making. A flow cytometric scoring system (FCSS) based on immunophenotypic aberrances in the (im)mature myelo-monocytic lineage and number of blasts enables identification of prognostic subgroups of patients with MDS (Wells et al. Blood 2003, Scott et al. Blood 2008, van de Loosdrecht et al. Blood 2008, Chu et al., Leuk Res 2011). In International Prognostic Scoring System (IPSS) low and intermediate 1 risk patients with MDS who are treated with growth factors, an increased endogenous erythropoietin level combined with presence of aberrant myeloid progenitors is predictive for response failure (Westers et al., Blood 2010).

Although there are clear indications that FC is of added value for the clinical practice of MDS, its use is not yet widespread. We aimed to implement FC in our centre by following European LeukaemiaNet guidelines for sample handling, to assess the prognostic relevance of FC and specifically the FCSS for MDS (van de Loosdrecht et al., Haematologica 2009, Westers et al., Leukemia 2012).

Bone marrow samples of 153 patients diagnosed with MDS by morphology and/or cytogenetics were processed and analysed by FC within 24 hours of the sample being drawn as per European LeukaemiaNet guidelines. As a control population, BM samples of age-matched pathologic controls with confirmed non-myeloid clonal disease were included, as well as 37 age matched healthy controls as a reference population. Each individual was given a FCSS score and scores were categorised into 0 points as normal to minimal flow cytometric aberrances, 23 points as mild and ≥4 points as severe flow cytometric aberrances.

**Results**

The median FCSS of patients with MDS was 4 and significantly higher than the FCSS of pathologic and healthy controls, median FCSS=1 in both groups, p<0.001 compared with both groups. There was a significant correlation between the FCSS and IPSS, Pearson r=0.44, p<0.001. Patients with MDS and a mild FCSS had significantly better overall survival (OS) compared with patients with severe FCSS, median OS 50.3 vs. 17.1 months, p=0.008. However, the number of patients with minimal flow cytometric aberrancies in myelopoesis was low and survival was not significantly different from patients with mild aberrances. The median FCSS was 1 in the pathologic and healthy control group. Therefore, we adjusted the cut points for the FCSS into 0-2 points for minimal flow cytometric aberrances, 3-4 for mild and ≥5 for severe aberrances. Patients with 0-2 points had significantly better OS than patients with 3-4 points, median OS 65.2 months vs. 40.6 months, respectively, p=0.04.

**Conclusions**

It can be concluded that flow cytometric analysis of BM from patients with MDS can identify distinct prognostic subgroups. The implementation requires further optimisation, which is one of the assignments of the European LeukaemiaNet working party for FC in MDS.

**Background**

miRNAs are small, non coding RNAs involved in the regulation of biological processes, primarily through interaction with messenger RNAs. The expression of free circulating miRNAs in peripheral blood has been shown to be associated with various neoplastic conditions. It is in discussion if these miRNAs can be potential biomarkers in different pathophysiological conditions. More than 1500 miRNAs are involved in post transcriptional regulation and have been described and linked to the onset of acute leukemia and MDS. Recently we have described a profile of 15 miRNAs detected in complete peripheral blood of MDS (PE-04581). The aim of this study is to analyze if the miRNA profile defined in peripheral blood in MDS patients will be reproduced in cell free plasma miRNAs.

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**Methods**

We have performed an initial study in 40 patients diagnosed with MDS in our department (2008 2011). Differential expression levels of miRNA versus a control group of 40 healthy subjects matched for age and sex were analyzed and identified in peripheral blood samples deposited in the Aragon Biobank. Screening to identify a miRNA signature associated with prognosis in MDS was determined by quantitative real time PCR using Megaplex™ Primers Human Pool AV2.1 and B (P/N 4399966 and 4399968, Applied Biosystems, USA), respectively. The kit contained assays for 754 miRNAs of the 1000 currently listed in the Sanger miRbase database (Griffiths-Jones et al., 2006). PCR reactions were performed using a ViiA7 Real Time PCR System. A profile of 15 differentially expressed miRNAs was selected. To obtain cell free plasma miRNAs, we used the “Total RNA Purification Kit” (Norgen, Canada) according to manufacturer’s instructions. A previous step of cDNA pre amplification was performed with Megaplex Pre Amp Primers Human Set (Applied Biosystems, USA).

**Results**

For 40 patients we analyzed the correlation with the differentially expressed miRNAs in complete peripheral blood and plasma. Mean age: 67 years (19-86), M/F: 19/21, IPSS distribution low risk (AR/AR/SMD/AREB 1/54/19/3), high risk (RCMD/AREB 2/LAM SMD): 11 patients (4/7/2), 5q (-): 4 patients, 7q (-): 4 patients. Fifteen miRNAs demonstrated a different expression in SMD patients vs. healthy people: miR-625-5p, miR-625-3p, miR-24-3p, miR-140-3p, miR-361-3p, miR-19b-3p, miR-942, miR-15b-5p, miR-378a-3p, miR-99b-5p, miR-26a-5p, miR-378a-5p, let-7e-5p, miR-16-5p, and miR-451a. The miRNAs’ concentrations in plasma samples compared with the complete peripheral blood were significant lower.

**Comments**

Our results need further analysis, increasing the number of samples and considering others co morbidities to determine their clinical significance.
Objective and Background
Loss of the Y chromosome in a proportion of blood cells of elderly men is a normal event and not disease related. However, this abnormality is also detected in patients with myelodysplastic syndromes (MDS) where it is still a matter of debate whether it is an age-related phenomenon or a clonal abnormality. Noteworthy in MDS patients is that the prognosis of an isolated Y loss is better than that of a normal karyotype, although the mean age of MDS patients with isolated Y loss is higher than the typical MDS cohort. In the present study we aimed to analyze the impact of age and disease on the occurrence of Y loss in MDS.

Methods
We assume that a disease related Y-loss would be present in CD34+ clonal cells, but not in CD3+ T cells not belonging the clonal area. Thus, we determined the frequency of cells with Y loss in CD34+ and CD3+ cells of younger (<35 years) and elderly (>60 years) MDS patients and control persons not suffering from hematopoietic diseases. CD34+ and CD3+ cells were enriched by magnetic (MACS) or fluorescence (FACS) activated cell sorting. The frequency of cells with Y loss was determined by fluorescence in situ Hybridization (FISH) analysis. We analyzed CD3+ and CD34+ peripheral blood cells (PBC) of 25 MDS patients (median age (MA) 78 years) and CD3+ PBC of 25 younger control persons (MA 27 years). In addition, CD3+ PBC of 27 elderly control persons (MA 74 years), CD34+ PBC of 10 elderly control persons (MA 74 years) and CD34+ and CD3+ bone marrow cells of four elderly control persons (MA 76 years) were analyzed so far.

Results and statistical significance
In elderly MDS patients, the number of cells with Y loss was significantly increased in CD34+ cells (median clone size 47%, range 8%-97%) compared to CD3+ cells (median clone size 6%, range 0%-14%, p<0.01). We could not include younger MDS patients as MDS is extremely rare in this group. The median frequency of Y loss in CD34+ cells of healthy young men was 0.5% (range 0%-2%), compared to 2% (range 0%-14%) in CD3+ cells of elderly control persons (p>0.01). The Y loss observed in 9% (median) of CD34+ PBC (range 2%-14%) of elderly control persons was significantly lower than it was in elderly MDS patients (p<0.01). However, there was no significant difference in the clone size between CD3+ PBC of elderly control persons and MDS patients (p>0.01). To date, the analysis of CD34+ PBC of young healthy men is pending.

Conclusions
The detection of Y loss in CD3+ and CD34+ cells of elderly control persons implies an age related Y loss in both cell types. However, the increased frequency of Y loss in CD34+ cells of MDS patients also suggests a disease related component. Therefore, we conclude that the Y loss in MDS patients is not just age related or clonal but often a combination of both. We now aim to establish an age matched laboratory threshold for Y loss in CD34+ cells to improve diagnostic accuracy.

[44] Loss of the Y Chromosome Results From a Combination of Age- and Disease Related Factors in Patients with Myelodysplastic Syndromes

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[45] Detection of Cryptic Chromosomal Alterations in Patients of the AZALE Study

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Objective and Background
The 20 high-risk MDS and sAML patients included in the AZALE study were treated with lenalidomide in combination with 5-azacitidine. To identify patients who respond well to the treatment regimen, numerous biomarkers that could affect therapy were analyzed. Amongst others, chromosomal aberrations were detected by conventional chromosome banding (CCB) in all patients. However, cryptic chromosomal abnormalities not detectable by CCB, such as micro-deletions, duplications/amplifications and regions with loss of heterozygosity (uniparental disomies, UPD), could also be associated with treatment response. Therefore, we aimed to detect them using molecular karyotyping by high-resolution SNP array analysis (SNP A).

Methods
This interim analysis takes SNP A results of 9/20 patients included in the AZALE trial into account. Seven patients were analyzed before or after the first cycle of treatment (3 RAEB2, 2 AML, 2 sAML), one patient (RAEB2) on day 119 and another one (RAEB2) on day 56 and 160 after first cytogenetic screening for the study. DNA was isolated either from immuno-magnetically enriched CD34+ peripheral blood cells (6 samples), CD34+ bone marrow cells (2 samples), or cytogenetic preparations (2 samples). SNP A was performed with arrays from Affymetrix (4x SNP 6.0, 6x CytoScanHD). Results from CCB and fluorescence in situ hybridization (FISH) analysis were available for all patients.

Results and significance
Cryptic chromosomal abnormalities were identified in those 5 patients with known complex aberrations, but also in all 4 patients with ≤3 abnormalities detected by CCB and/or FISH A. A patient with 16 abnormalities detected by CCB and SNP A developed under treatment four additional micro-deletions (del(2q)(13q12), del(11q)(14q24q26)) within 104 days, indicating clonal evolution. Even the detection rate of common chromosomal aberrations could be increased by SNP A. The del(5q) that was required to be included in this study was confirmed in all 9 patients by FISH and SNP A (minimal deleted region (MDR): 5q23.1q31.3, 15.1 Mb). Chromosome 17 abnormalities were identified by SNP A in 7/9 patients, upd(17p) in two patients and del(17p) including TP53 in 5 patients. This includes one previously unknown del(17p) where the FISH probe overlapped with the breakpoint of a 0.487 Mb micro-deletion in 17p13.1. This micro-deletion was also the MDR in these 5 patients. By FISH- and SNP A 4/9 patients showed a del(12p) including TEL/ETV6 (MDR: 12p13.2p13.1, 1.1 Mb). A del(4q) including TET2 was identified in 3/9 patients by FISH-A and SNP A (MDR: 4q24q26, 14.9 Mb), while in just one of these patients del(4q) was detected by CCB, due to an insufficient number of metaphases or the small size of the deletion (14.9 Mb).

Conclusions
We could show that molecular karyotyping of high risk MDS patients helps to characterize these patients more accurately. Analysis of further patients might allow identifying chromosomal regions that are associated with response to therapy. The information gained could be used for therapy decisions. Particularly, aberrations that occur in the course of the disease (karyotype evolution) could be associated with progression and might help to identify new therapeutic targets.
[46] Analysis of Efficacy and Safety of Two Iron Chelators in Patients with Iron Overload (QueLafer Study)

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Background
Iron excess in blood and tissues causes irreversible tissue damage. It has been demonstrated that removal of excess iron has a positive influence on the response to treatment and survival in patients with overload. Aims: To evaluate the efficacy and the safety of two iron chelators (intensity and time to response), and the presence and frequencies of adverse events and quality of life (QOL). Some biomarkers of macrophages (chitotriosidase and CCL18/PARC) could be accurate indicators of tissular iron overload.

Design and patients
Comparative, randomized, open, non-inferiority experimental study in 27 patients with maintained iron overload ( ferritin>500 µg/L), SMD/LMA (13) iron overload posttransfusion, allo-BMT(8) and type 1 Gaucher Disease (6), were compared with a healthy control group of 27 subjects stratified by age and sex. The study was approval by the Ethical Committee of Aragon and supported by a grant (TRA-158 EUDRACT: 2009-017799-26). All participants signed informed consent. A baseline study: physical examination, cell counts, iron and protein profiles, proBNP, chitotriosidase and CCL18/PARC, HFE gene, heart function, iron liver deposits (LIC) by MRI and evaluation of quality of life by SF36 questionnaire. Two randomized groups: A: Deferasirox 20 mg/Kg/day p.o. and B: Desferoxamine: 30 mg/Kg/day sc for 8 hours, three times a week. Treatment period: 4 months. Patients were monitored weekly in the first 4 weeks and every month thereafter.

Results
Mean age: 56.9 y (29-77), 52% female. 30% of patients were heterozygous for H63D and one was homozygous. Mean at baseline; Hb: 12.4 g/dl (7.2-15.2), Hematocrit: 36.3% (20-45.2), Ferritin: 1042.8 ng/mL (635-1461), ProBNP: 381.1 pg/mL (18.8-2804), LIC: 83.2 µmol/g (0-240), Chitotriosidase: 65.2 mmol/L (0.81-186) and CCL18/PARC: 150.3 ng/mL (56-393). Fifteen patients were included in group A and twelve in B. Eight patients required transfusion of packed red cells during the study period (mean; 3 units).

After 4 months on therapy, 80% of patients showed a significant reduction in ferritin levels in both groups A: 490.7 ng/mL (155-1090) (p<0.001), B: 662.7 ng/mL (312-1395) (p<0.05), the differences in reduction grade were similar in the two series, proBNP 368 pg/mL (30-2705), LIC: 50.6 µmol/g (0 190). According the diagnosis, the patients with GD have a baseline ferritin levels significantly lower than the other groups (p<0.04) but the grade of reduction after therapy was similar. Adverse events: In group A, three patients experienced a reversible mild increase in creatinine levels; one of them was grade 3 and mild digestive intolerance in other 3 cases. Related to QOL, group A patients showed significantly better scores in the mental component scales (p<0.03) than group B.

Conclusions
• Significant decreases of ferritin and iron liver deposits with both chelators were observed after four months of therapy. No significant relationship were observed between ferritin decrease and plasma biomarkers of activated macrophages.
• Safety: Few adverse effects. Only patients in oral therapy had gastrointestinal disturbances and slight and reversible increase of creatinine levels in the first weeks.
• Quality of life: No statistical differences were observed in physical component scores between groups A and B, nevertheless, patients under oral therapy have significant improvement in mental component scores.

[47] Hypomethylating Therapy with Decitabine Prior to Allogeneic Stem Cell Transplantation for MDS/AML Patients

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Background
Decitabine (DACogen) (Dac) is a hypomethylating agent with activity in myelodysplastic syndromes (MDS). Novel non-intensive treatment options in MDS patients (pts) planned for alloHSCT with the goal of down standing disease and increasing time to transplantation are presently being developed. It is unknown whether treatment with the drug before alloHSCT will increase the toxicity of the preparative regimen.

Materials and Methods
We analyzed the outcome of 12 MDS/AML pts (median age 33, range 9 54 years) who underwent alloHSCT from matched sibling (n=3), haploidentical (n=3) or unrelated (n=6) donors after prior therapy with Dac. At diagnosis 9 pts had high risk MDS by international prognostic scoring system. Poor risk cytogenetic (monosomy 7, complex or chromosome 3 abnormality) had 8 pts. The pts received Dac 20 mg/m2/day on days 1-5, 28 day cycle with a median of 4.7 cycles (range 1-9) and a median duration of treatment of 6.3 months (range 1 13). Disease status at the time of transplantation was: partial remission (2 pts), stabilization (8 pts) and progression (2 pts). Fludarabine based reduced intensity conditioning regimen (RIC) received 10 pts, myeloablative regimens (MAK) (Bu+Cph) – 2 pts. Prophylaxis of acute GVHD was performed by a tacrolimus or cyclosporine A based regimen and methotrexate (short course). In a case of unrelated alloHSCT horse antithymocyte globulin was used at a dose of 60 80 mg/kg for GVHD prophylaxis. The source of stem cells was bone marrow, peripheral blood and both in 3, 5 and 4 cases, respectively. Mean CD 34+Kg cells count was 4.4 x106 cells (range 1.0-9.7).

Results
Dac was well tolerated without severe complications. Engraftment confirmed in 10 pts, two had primary engraftment failure, and one, graft rejection. Mean time to bands and platelet engraftment was 19.5 days (range, 12-32) and 16 days (range, 11-32) respectively. Acute GVHD (grade II) developed in 3 pts (25%), and chronic GVHD (extensive form) in 3 pts. At day +100: 7 pts had full donor’s chimerism, 2 pts had mixed chimerism, 2 pts had primary engraftment failure, and one patient developed thrombotic thrombocytopenic purpura (TTP) (died at day +58). Within 100 days after alloHSCT 3 pts were in CR. With a follow up of 19 months (range, 2-19) 5 pts are alive: 3 in CR, 2 in PR (1 after the second alloHSCT). 7 pts died (1 PD, 3 chronic GVHD, 1 TTP, and 2 graft from failure). No TRM on day +100 was in patients after related alloHSCT. One year OS was 50%.

Conclusion
The prior therapy with a hypomethylating agent Decitabine is feasible and allows the majority of pts with MDS to achieve remission before alloBMT. This drug shows no unexpected toxicity, severe aGVHD decreases TRM, does not increase complications after transplantation and appears to be a part of pre transplant strategy in MDS/AML patients.
MDs

Skewed X-Inactivation Patterns in Aging Healthy and Myelodysplastic Hematopoiesis Determined By a Novel Transcriptional Clonality Assay

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Objectives and Background

X-chromosome inactivation is a well described process that occurs randomly during human female embryogenesis in order to maintain gene dosage equivalence between male (XY) and female (XX) cells. Investigation of X-chromosome inactivation patterns (XCIP) by determination of differential CpG methylation has been widely applied for investigation of female cell clonality. Using this approach the clonal origin of various tumours has been corroborated via detection of skewed XCIPs. Controversially, a strong age related increase of peripheral blood (PB) cell clonality in hematologically healthy females was reported. Recently, transcriptional XCIP ratio analysis challenged these results and questioned the suitability of methylation based clonality assays. To reinvestigate XCIP skewing in hematopoiesis of healthy females and patients with myelodysplastic syndrome (MDS), we established a novel transcriptional assay utilizing a pyrosequencing technique for quantification of SNP allele frequencies, representative for XCIP ratios.

Methods: BM, CD34+, PB cells and granulocytes were obtained from patients with MDS (IPSS:low/int-1-risk BM: n=25, CD34+: n=13, PB: n=21, IPSS: int-2/high risk: BM: n=16, CD34+: n=9, PB: n=12) and age related healthy donors (BM: n=19, CD34+: n=15, PB: n=154, granulocytes: n=34; age range: 0-97) after informed consent. Genomic DNA SNP genotyping was carried out in order to screen for heterozygous XCIP marker genes located on the X chromosome, namely BTK, FH11, IDS, MPPI and G6PD. For assessment of XCIP skewing we developed pyrosequencing assays for each marker gene. After PCR amplification of informative marker loci from cDNA transcripts, SNP allele ratios, representative for XCIP, were quantified using the PyroMark ID system (Qiagen, Hilden, Germany). Skewing was assumed for samples exhibiting skewed XCIP ratios >80% (allelic ratio of >4:1).

Results

Standard curves from pyrosequencing reactions with predefined allelic ratios revealed strong correlations for assessment of XCIP ratios in all markers (R²>0.99). Furthermore, high correlations have been detected for inter-marker XCIP ratios from individuals with multiple informative markers ranging from R²>0.83 to R²<0.98 as well as for marker results between matched PB and granulocytes (R² = 0.95-0.99) emphasizing the suitability of the method. XCIP skewing incidences were strongly elevated in PB cells of hematologically healthy old females (mean: 51%, age >65 years) compared to young females (mean: 14%, age <40 years, p=0.0002). MDS patients exhibited strongly increased skewing incidences of 90-100% in CD34+, BM and PB cell fractions when compared to healthy old and young females with 43-44% and 20-25% in the BM and CD34+ compartment. Excessive skewing (XCIP ratios >95%) was frequently detected in 50-67% of all MDS cell types and almost absent in healthy specimens except for BM and CD34+ cells of healthy old females (22-29%).

Conclusions

Significant age related increase of XCIP skewing in PB cells from healthy elderly females was confirmed. Moreover, XCIP ratio analysis suggests even stronger clonal manifestation in aged BM and CD34+ cells. In MDS, XCIP skewing levels were distinctive ly elevated as compared to age matched controls and higher degrees were associated with poor clinical outcome. In conclusion, our pyrosequencing based XCIP analysis approach allows accurate assessment of XCIP ratios, reveals novel insights into aging healthy and myelodysplastic hematopoiesis, and should be easily applicable to other fields of research.
[49] Assessment of Transfusion Related Cardiac and Liver Iron Overload in Child Patients with MRI T2* Technique: Single Centre Initial Experience

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Objectives and Background
Research of the application of MRI T2* to assess transfusion related cardiac and liver overload level in children patients. Iron deposition disrupts the homogenous magnetic field and shortens tissue T1 and T2 times in a concentration-dependent manner. MRI is the only non invasive method for indirect assessment of myocardial iron. Myocardial T2* values <20 ms are considered below the normal lower threshold and are associated with a progressive and significant decline in left ventricle ejection fraction (LVEF) and arrhythmias. Several studies calibrated liver T2* measurements with liver biopsy samples although some report T2* may not be accurate at very high liver iron content. We aimed to gather more clinical and MRI data on child patients as most published studies are based on adults.

Patients and Methods
8 patients (aged 7-15 y) received multiple blood transfusions (due to AML, MDS etc.) and were prospectively evaluated using 1,5 T MRI (Avanto, Siemens) along with clinical data (serum ferritin, UIBC etc.) in Vilnius University Hospital Santariskiu Klinikos during 2011-2012. No myocardial and liver biopsies have been made so far. MRI T2* technique was used for all patients to evaluate heart tissue iron overload. A single cardiac/liver image was obtained during 1 breath-hold (easier for patient). We used 8 different echo times per image for heart and 12 for liver. T2* time was calculated from gradient echo images using CMRtools 2010 (© Cardiovascular Imaging Solutions). Cardiac T2* was calculated from intraventricular septum region (represents both ventricles) in short-axis view. Liver T2* calculation included ROI’s of both lobes in transversal plane. Left ventricle ejection fraction was evaluated from short axis images using Argus software (Siemens). Contrast medium is not required for iron overload assessment. All patients successfully underwent the MRI procedure without need for general anaesthesia (respiration and motion artefacts were not significant). Total MRI procedure time ranged 20-30 min and all patients found it comfortable.

Results
Clinically significant iron overload in myocardium was excluded in all 8 patients validating T2* against cardiac function: myocardial T2* was >20 ms (20.6-52.2) and EF >50 %. 1 patient had T2* lower normal threshold value of 20.6 ms and will be clinically monitored for possible development of arrhythmias and LVEF decrease. Liver T2* values ranged 1.9-12.4 ms and according to Tucci et al. (2008) 3 patients were suspected to have moderate and 2 patients mild iron overload in liver.

Conclusions
MRI T2* technique can be applied to assess transfusion-related cardiac and liver overload level in children patients, but more data (patients) is needed to determine its clinical and prognostic value. Preliminary results of iron overload assessment by MRI T2* in 8 patients tend to correlate with transfusion load, but more data (patients) is needed to assess for any statistically significant correlation with clinical data (serum ferritin and UIBC, LVEF, arrhythmias etc.) with the aim of avoiding liver and myocardium biopsies in young patients. General anaesthesia is likely to be avoided for heart and liver MRI in order to assess iron overload in children aged 7 and above.

[50] Systemic Iron Overload as Measured by MRI is an Independent Prognostic Factor in AML and MDS Patients Undergoing Allogeneic Stem Cell Transplantation

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Introduction
Inconsistent associations between systemic iron overload (SIO), as defined by surrogate parameters like serum ferritin or transfusion burden and outcome have been reported in patients undergoing allogeneic stem cell transplantation (allo-SCT). In fact, the specificity of these surrogates as well as their thresholds for risk stratification remains under debate.

Methods
The correlation between pre transplant magnetic resonance imaging (MRI) based liver iron content (LIC) and surrogate parameters as well as the impact of SIO on post transplant outcome was assessed prospectively in a cohort of AML (n = 64) and MDS (n = 24) patients.

Results and Significance
Ferritin levels ≥1000 ng/ml provided only poor specificity (31.8 %) for predicting elevated LIC (≥125 µmol/l). Neither surrogate parameters nor LIC correlated with an increased risk of bacterial infections or acute Graft versus host Disease. Moreover, there was no association between ferritin or transfusion burden and Non Relapse Mortality (NRM). In contrast, a LIC ≥125 µmol/l was a significant risk factor for NRM in uni and multivariate analysis (HR = 2.98, p=0.016). Multivariate Cox regression also showed that LIC >125 µmol/l was associated with a decreased OS (HR = 2.24, p=0.038), whereas ferritin or transfusion burden were not.

Conclusion
We conclude that SIO reflected by LIC is an independent negative prognostic factor for post transplant outcome in AML and MDS patients undergoing allo SCT and should be preferred to surrogate parameters for patient selection in upcoming clinical trials on iron specific therapeutic interventions.
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