632 Chronic Myeloid Leukemia: Therapy: Response Monitoring and Prognosis

Monday, December 9, 2019: 10:30 AM-12:00 PM

W308, Level 3 (Orange County Convention Center)

Moderators:

Elisabetta Abruzzese, MD, Hematology Unit, S. Eugenio Hospital, Tor Vergata University and B. Douglas Smith, MD, Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins

661 Detection of Actionable BCR-ABL1 Kinase Domain (KD) Mutations in Chronic Myeloid Leukemia (CML) Patients with Failure and Warning Response to Tyrosine Kinase Inhibitors (TKIs): Potential Impact of Next-Generation Sequencing (NGS) and Droplet Digital PCR (ddPCR) on Clinical Decision Making

Simona Soverini, PhD

A variety of mechanisms underlie the lack or loss of response to TKIs in patients (pts) with CML, but acquisition of point mutations in the BCR-ABL1 KD is, at present, the only actionable one. Detection of a TKI-resistant mutation by Sanger Sequencing (seq) should trigger a change of therapy and, in some cases, guide TKI selection. Although research studies have shown that NGS may hold added value over Sanger seq for BCR-ABL1 KD mutation screening, routine NGS is technically demanding and expensive and is not yet widely available. Newer methods, like ddPCR, might represent an attractive alternative. Here we set out to 1) assess the actionability of results, hence the potential clinical benefit, of more sensitive NGS-based testing vs Sanger seq in a consecutive series of CML pts who had non-optimal response to TKI therapy according to the 2013 ELN recommendations, and 2) test a novel ddPCR-based multiplex assay for rapid screening for a panel of BCR-ABL1 KD mutations relevant for TKI selection.

Between January 2015 and May 2019, samples from 712 CML pts followed at one of 66 GIMEMA CML Working Party hematology centers were referred to our laboratory for BCR-ABL1 KD mutation testing because of a Failure (n=251 pts) or Warning (n=461 pts) response to TKI therapy. In parallel to Sanger seq, NGS of amplicons generated by nested reverse transcription(RT)-PCR was performed on a Roche GS Junior instrument until April 2017, and on an Illumina MiSeq instrument from May 2017 on. Read alignment and variant calling was done using AmpSuite.
software (SmartSeq). Variants detected in <3% of BCR-ABL1 transcripts were filtered out. ddPCR was performed on a QX200 instrument using a single-tube assay (BioRad) containing primers and probes for the simultaneous detection and discrimination of 2nd generation TKI (2GTKI)-resistant mutations: T315I/A, Y253H, E255K/V, F359V/I/C, V299L and F317L/V/I/C. Data analysis was performed using QuantaSoft software.

Among Failures, pts positive for BCR-ABL1 KD mutations by Sanger seq were 88/251 (35%). NGS detected low level mutations in 38/251 (15%) additional pts who were negative for mutations by Sanger seq. Moreover, 31/251 (12%) Failure pts were found to have low level mutations additional to those detectable by Sanger seq. Among low level mutations detected by NGS, those actually relevant for TKI selection (2GTKI-resistant) were identified in 22/251 (9%) pts. Compound mutations were found in 10 pts (4%; all progressed to blastic phase). The average turnaround time (TAT) of routine NGS-based analysis was 15 working days (range, 11-32). Thus, in the setting of Failure, NGS-based mutation scanning of the whole KD is of real clinical benefit in a minority of pts while increasing the TAT.

Among Warnings, pts positive for BCR-ABL1 KD mutations by Sanger seq were 65/461 (14%). NGS detected low level mutations in 97/461 (21%) additional pts who were negative for mutations by Sanger seq. All Warning pts positive for low level resistant mutations who were not switched to another TKI turned their response into Failure after 3 to 8 months.

The multiplex digital PCR assay proved capable to accurately identify and separate the T315I/A, F317L/V/I/C, Y253H, E255K, F359V/I/C, E255V, V299L mutations in five spatially distinct clusters (pan-resistant, Dasatinib-resistant, Nilotinib-resistant, Nilotinib- and Bosutinib-resistant, Dasatinib- and Bosutinib-resistant, respectively) starting from as little as 30ng of total cDNA or 1ng of the 1.7kb 1st PCR product. Very good concordance was observed between ddPCR- and NGS-identified mutations irrespective of mutation frequency or cluster proximity, even for compound mutations. Extensive assay assessment will be presented. TAT of ddPCR was 1 day.

In conclusion:

- Mutation testing in the Failure setting aims to a timely and rational TKI switch. The incidence of low level mutations relevant to the selection of subsequent-line therapy and the TAT of routine NGS in our cohort suggest that multiplex ddPCR would be an easier and faster alternative.

- Mutation testing in the Warning setting may identify pts who need a change in therapy rather than a ‘watch and wait’ approach. In our cohort, approx 1/5 of the Warning pts negative by Sanger seq had low level mutations resistant to the TKI they were receiving, that ultimately led to Failure. Earlier detection of emerging resistant mutations enabled by NGS (or by ddPCR in pts receiving 2GTKIs) should support proactive TKI switch.
Figure 1: Graphical output of the single-tube multiplex ddPCR assay tested. Left panel: expected configuration of the fluorescence clusters corresponding to the five mutation subgroups and to p190 and p210 wild-type transcripts. Right panel: representative 2D plot obtained for a TKI-resistant sample carrying the E255K mutation. Overlay with clusters generated by five positive controls run in parallel (not shown) enables to determine the second-generation TKI-resistance profile: in this example, droplets fall in the first cluster, thus the sample is positive for a mutation that is resistant to nilotinib and bosutinib (E255K). For each plot, the amplitude in channel 1 (Ch1) is represented on the y-axis and the amplitude in channel 2 (Ch2) is represented on the x-axis. Black: empty droplets; blue: mutant cDNA positive droplets; green: wild-type cDNA positive droplets; orange: wild-type and mutant cDNA double positive droplets. Abbreviations: DAS, dasatinib; NIL, nilotinib; BOS, bosutinib; res, resistant.

<table>
<thead>
<tr>
<th></th>
<th>No. of pts with BCR-ABL1 KD mutations when using Sanger seq</th>
<th>No. of pts with BCR-ABL1 KD mutations when using NGS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any</td>
<td>2GTKI-res</td>
</tr>
<tr>
<td>Failures</td>
<td>88/251 (35%)</td>
<td>31/251 (12%)</td>
</tr>
<tr>
<td>Warnings</td>
<td>65/461 (14%)</td>
<td>22/461 (5%)</td>
</tr>
</tbody>
</table>

Table 1: Breakdown of pts positive for any mutation or for 2nd-generation TKI (2GTKI)-resistant (res) mutations only (those that impact on TKI: T315I/A, F317LV/IC, Y253H, E255K, F359V/IC, E255V, V299L) by Sanger-sequencing and by NGS (with 2GTKI-resistant mutations also detectable by the BioRad multiplex ddPCR assay). In our experience, in the Failure setting, NGS brought a not negligible increase in TAT against a modest increase in the number of pts found to harbor mutations relevant for TKI selection. In the Warning setting, NGS was capable to identify emerging TKI-mutations below the lower detection limit of Sanger seq in 21% of the pts. Whenever the pts were not switched to another TKI, response later turned into Failure – suggesting that sensitive mutation testing may be beneficial in the Warning setting. Besides representing an indication for treatment change, in one third of the cases the low level mutation was also impacting on subsequent-line TKI selection (and was also detectable by the multiplex ddPCR assay).
662 RNA Splicing Defects in Cancer-Linked Genes Indicate Mutation or Focal Gene Deletion and Are Associated with TKI Resistance in CML

Naranie Shanmuganathan, MBBS FRACP FRCPA

Background: Mutated cancer genes in patients (pts) with TKI failure and blast crisis (BC) CML have recently been described. RUNX1 mutations, namely single nucleotide variants (SNVs) and indels, were the most frequently detected besides BCR-ABL1 [reviewed in Branford, Kim Leuk 2019]. They were found in ~18% of pts, although splice variants were rarely described. RNA splicing events were associated with focal deletion of IKZF1 and RUNX1 in TKI resistant pts that were identified by copy number analysis and RNAseq [Branford Blood 2018]. Novel splicing associated with mutation of cancer genes is an unexplored area of study in resistance. RNA sequencing can assess the functional effect of splice site variants, which lead to splicing errors due to the use of alternative or cryptic splice sites and cause alterations to protein function.

Aim: We determined whether novel splicing can identify cancer genes with potential altered function.

Methods: RNAseq analysis was performed for 48 pts at diagnosis and 33 at BC using a protocol that preserved intron-retaining precursor RNA. Coverage of intron-exon borders was sufficient to detect intronic splice region variants. The STAR aligner was used to bioinformatically collate unannotated RNA splice junctions. 54 cancer genes were assessed and aberrant splice events were filtered based on the number of samples in which a splice junction occurred. Manual inspection of the splice junctions was performed using the Integrative Genomics Viewer. This approach identified previously verified aberrant splicing associated with IKZF1 and RUNX1 deletions.

Results: Ten previously undetected novel splice junctions were revealed in 9/33 pts (27%) in BC within key tumor suppressor genes CDKN2A/B (5), RB1 (1), ATM (1), and RUNX1 (3).

The aberrant splicing pattern of CDKN2A and RB1 (Fig A/B) in 6 pts suggested large deletions, as previously described in our cohort with IKZF1 and RUNX1 deletions. Breakpoints associated with deletions ranging from 53 to 181 Kb were detected in the 5 pts with CDKN2A aberrant splicing. Similarly, a 90 Kb deletion of exons 18-27 of the RB1 gene led to the aberrant splicing. The pts transformed to lymphoid BC (median 5 months). 4 of these 6 pts were tested at diagnosis and the deletions were not detected, indicating they were gained at resistance.
The aberrant splicing patterns of ATM and RUNX1 did not predict large deletions. These were related to somatic SNVs at canonical splice sites in ATM and in 2 of the pts with RUNX1 aberrant splicing. A splice acceptor site SNV in ATM resulted in skipping of exon 61 (Fig C) and protein truncation. This novel SNV has not been reported in any population or somatic variant database.

Two pts in myeloid BC at 28 and 48 months after diagnosis had an identical somatic RUNX1 mutation at the canonical splice donor site of exon 5. The SNV was not detectable prior to imatinib treatment in both pts. The splice site SNV led to activation of a cryptic splice site within exon 5 in both pts (Fig D), which predicted premature termination. While this mutation is novel, an adjacent intronic SNV occurs in familial platelet disorder, leading to activation of the same cryptic splice site.

The atypical RUNX1 splicing of the 3rd patient was associated with retention of 55 bp of intron 6 as a cryptic exon (Fig E), leading to protein truncation. A deep intronic SNV identified at lymphoid BC at 6 months of imatinib was detected near the cryptic exon by RNAseq and verified as somatic by DNA Sanger sequencing. This was predicted to activate cryptic RNA splicing elements and lead to intron sequence retention in a RUNX1 transcript. We sequenced the diagnosis sample using an RNA-based gene panel method under development that provides enhanced sensitivity of variant detection. The same pattern of atypical splicing was observed and the intronic SNV was present at low level. The RUNX1 mutation at diagnosis may have contributed to early BC. To our knowledge this is the first report of a RUNX1 truncating variant in CML involving a cryptic exon.

**Conclusion:** Enhanced bioinformatic analysis of RNAseq data has revealed a high proportion of pts with truncating mutations in cancer genes indicated by novel RNA splicing (27% pts in BC). Using RNA-based sequencing allows an evaluation of the potential functional effect of variants that are not apparent by DNA-based mutation analysis. We suggest that future studies include RNA sequencing to detect a broader spectrum of mutations associated with TKI resistance.
Figure

Sashimi plots enable visualization of RNA splice junctions and can reveal atypical RNA splicing related to sequence deletion or splice site disrupting mutations. Arrows represent splice junctions connecting exons (including cryptic exons).

A. GENE DELETION: Sashimi plots demonstrating atypical RNA splicing associated with deletions of CONEXINS.

B. GENE DELETION: Sashimi plots displaying splicing from exon 17 of FAK.

C. SPLICE DISRUPTING MUTATION: Atypical splicing of ATM resulting in exon skipping.

D. SPLICE DISRUPTING MUTATION: Sashimi plot showing SWI disrupting splicing of exon 5 in RUNX1.
Introduction and Aim. Achievement of deep molecular response (DMR) is the prerequisite for treatment-free remission in chronic phase CML (CP-CML) patients (pts). Pts who fail to achieve early molecular response (BCR-ABL1 > 10% IS) at 3-months (mths), or have high ELTS score at diagnosis have inferior achievement of DMR. We and others have shown that the levels of NK-cell, T-cell, myeloid-derived suppressor cell, and neutrophils in the blood at diagnosis have an impact on DMR achievement. We hypothesized that Cluster of Differentiation (CD) (cell surface marker) gene expression might provide a surrogate marker to characterize immune cell composition. We aimed to identify pts who had a low probability of achieving DMR by 5 years (yrs) by combining 3-mths BCR-ABL1% and CD gene expression. This may enable clinicians to determine whether an individual patient is on a pathway towards DMR and potentially TFR or should be considered for a different therapeutic approach if TFR is the eventual goal.

Methods. 119 blood samples from the imatinib-based TIDEL-II trial were subjected to transcriptomic microarray profiling. A total of 357 CD genes classified by the HUGO Gene Nomenclature Committee CD molecular gene group were assessed. We defined DMR as achieving MR4.5 (BCR-ABL1 < 0.0032%) at two consecutive time points. To construct a predictive model, the samples were randomly assigned to discovery and validation cohorts. Recursive partitioning and construction of a regression tree with tenfold cross-validation based on expression of 357 CD genes and 3-mths BCR-ABL1% were used as inputs in the discovery cohort. The performance was assessed based on accuracy of prediction of DMR by 5 yrs. The final model was validated using the independent validation cohort. All the analysis was performed using R statistical software.

Results. Clinical variables (age, gender, ELTS, 3-mths BCR-ABL1%, MMR, and MR4.5) were well matched in the discovery (n=60) and independent validation cohort (n=59). The predictive model was constructed using the discovery cohort to reveal two risk groups: poor-risk (PR, 15% achieving MR4.5 at 5 yrs, n=19), and good-risk (GR, 88% achieving MR4.5 at 5 yrs, n=41) groups (Figure 1A-B). This model classified PR group by BCR-ABL1 ≥ 7.5% at 3 months OR BCR-ABL1 < 7.5% at 3 months with high CD302 gene expression (≥7.9 log2 gene expression; top 15%) at diagnosis. GR group was defined as having both BCR-ABL1 < 7.5% and low CD302 gene expression (<7.9 log2 gene expression). These variables were chosen by the model based on accuracy performance in predicting DMR. CD302 is a C-type lectin receptor involved in cell adhesion and migration. It is expressed in myeloid populations as well as in blasts and leukemic stem cells (LSC) in AML. High expression of CD302 in PR pts may be a surrogate for increased LSC. The model was validated in the independent validation cohort. Pts identified as PR in the
validation cohort had significantly lower 5-yr MR4.5 achievement rate (14%, n=14) compared to those with GR (82%, n=45, p=0.0002, Figure 1C).

We asked whether using the more conventional BCR-ABL1 10% cutoff instead of 7.5% in our model would give similar results, but the performance in predicting long-term DMR achievement was inferior: Pts predicted as PR with this criteria had ~2x higher achievement of DMR (e.g. 26% vs 14% using 3-mths BCR-ABL1 10% vs 7.5% cutoff respectively).

ELTS score have been associated with the probability of DMR achievement. We compared the performance of ELTS in combination with 3-mths BCR-ABL1% by replacing CD302 gene expression with ELTS. The predictive accuracy was inferior. Pts with 3-mths BCR-ABL1 ≥7.5% OR BCR-ABL1 <7.5% with high/intermediate ELTS (PR-2) had about 3.3-3.5 fold higher DMR achievement rate than the PR group with CD302 in both discovery and validation cohorts (Figure 1D-E). In contrast, pts with 3-mths BCR-ABL1 <7.5% and low ELTS (GR-2) had approximately 1.1-1.2 fold lower DMR achievement rate than the GR group with CD302 in both discovery and validation cohorts (Figure 1D-E).

Conclusion. We have constructed a predictive model for DMR achievement for pts who receive optimised frontline imatinib therapy. This model performs better than combining ELTS and 3-mths BCR-ABL1%. We postulate that this predictive model could enable identification of poor risk pts at 3 mths who would benefit from intensified therapeutic approaches to obtain eligibility for TFR and potentially optimal clinical outcome.

![Figure 1](image-url)
Prospective Evaluation of ABL Kinase Domain Mutational Analysis By Next-Generation-Sequencing in Newly Diagnosed CP CML Patients Undergoing First-Line Treatment with Nilotinib Alone or Nilotinib + Pegylated Interferon-α2a in a Prospective Phase III Trial

**Hugues de Lavallade, MD, PhD**

The acquisition of ABL1 Kinase Domain (KD) mutations represent the most frequent resistance mechanism in CP-CML patients (pts) treated with tyrosine kinase inhibitors (TKI). Currently, the standard assay relies on a poorly sensitive technique, Sanger Sequencing (SS). Thus, the detection of these mutations using SS might be too late to trigger a timely treatment change. In a national phase III academic trial (PETALS, EudraCT 2013-004974-82), we evaluated prospectively the value of a more sensitive technique, Next Generation Sequencing (NGS) to detect KD ABL1 mutations in newly diagnosed CP-CML patients randomized to get nilotinib 600 mg/d for 6 years ± Pegylated-INF-α2a (Peg-INF) 45 µg/wk for 2 years in combination.

**MethodS:** Newly diagnosed CP CML pts ≤65 years were randomized 1:1 to get NIL 300 mg BID alone (M0 to M48, arm A) vs Peg-INF alone for 30 days (M1→M0) 30 mg/wk as priming, prior to NIL 300 mg BID + Peg-INF 30 µg/wk 2 weeks, upgraded to 45 µg/wk thereafter, for up to 2 y (M0 to M24, arm B) followed by NIL alone for 4 more years unless pts enter a treatment-free remission phase. In addition to KD mutational analysis performed by SS as per protocol, patients also had KD mutational analysis performed by NGS at M3, M6, M12 and 6-monthly thereafter until achievement of a stable MMR, regardless of response. NGS assay was performed as previously described (Kizilors et al. Lancet Haematol 2019).

**Results:** Two hundred pts were randomized (99 in A, 101 in B), of which 96 patients (51/99 in A, 45/101 in B, p=0.399) underwent a KD mutational analysis performed by NGS as part of this study. The remaining 104 patients are currently being screened and the full dataset will be presented. Among the 96 patients tested, there was no difference in the distribution between the 2 arms with respect to gender, age [median 45 years (18-66)] or risk factors distribution (p=0.862 and 0.328 for Sokal and ELTS respectively in patients tested at 3 months). The median follow-up of this cohort is 45.0 (33.2-58.7) months. By 12 months, 11 patients [8/51 (11.8%) in A, 3/45 (6.6%) in B] had developed a KD mutation. After only 3 months of TKI therapy, 3 patients were found mutated (Y253H 2 pts, T315I 1 pt), of whom 2 pts were only detected using NGS. At M6, a KD mutation was found in 8 pts [A: 7 patients, B: 1 pt, (p= 0.055), of which 6/8 were not detected by SS, due to either low level Variant Allele frequency (VAF, n=5) or low level BCR-ABL transcript levels (n=1). Y253H mutations were found in 4 pts, T315I in 2 pts and E255K in 1 pt. Consecutively to KD mutation identification, 6/8 patients lost their response and were withdrawn from study (1 pt with a Y253H detected at M3 progressed to advanced phase), while 1 pt lost MMR at last follow-up and another pt with a mutation sensitive to nilotinib achieved MMR. KD mutations were detected while...
pts were in optimal response at M6 [BCR-ABL <1% (IS)] in 5 pts (of whom 2 pts were in MMR) and in 1 pt in the warning category [BCR-ABL at 2.2% (IS) with a low level T315I at 2% VAF which became detectable by SS only at M18]. The remaining 2 pts had KD mutations detected at M3 and were in warning and failure respectively at M6 according to ELN 2013. A landmark analysis performed at 6 months showed that patients with KD mutations detected by NGS had a significantly worse event-free survival (EFS, according to ELN, Guilhot J. et al. Blood 2012) compared to pts without KD mutation [25% (8%-83%) vs 64.4% (51.9-80.1%) at 24 months, p=0.001 figure]. Although there were higher proportions of pts with high risk SOKAL and ELTS in the group with mutation, it did not reach statistical significance (p= 0.054 and 0.082 respectively).

Conclusions: This is the first prospective trial to demonstrate that NGS can detect low level KD mutations in CP CML patients treated with first line 2GTKI±Peg-IFN after only 3 to 6 months on therapy before these become detectable by SS and despite achieving an optimal response in BCR-ABL transcript level reduction (according to ELN 2013). The proportion of patients who develop KD mutations by 12 months on upfront 2GTKI should not be underestimated, as their outcome is poor. NGS may trigger early clinical intervention and prevent progression in this group, although a prospective trial is needed in this regard. Finally, the proportion of KD incidence in pts who receive Peg-IFN in addition to nilotinib might be lower compared to those treated with nilotinib alone. Final updated results will be presented.
Background: Achieving sustained DMR (variably described as ≥ MR4 or ≥ MR4.5) is an emerging treatment goal for patients with CML. DMR is associated with excellent long-term clinical outcomes and a higher likelihood of successful treatment-free remission (TFR) upon discontinuation of tyrosine kinase inhibitor (TKI) therapy. Biological predictors of patients likely to achieve DMR are unknown. Here, we present an exploratory analysis of gene expression signatures in order to predict DMR to TKI therapy, as well as understand the biological underpinnings that allow a DMR, based on patients treated with imatinib or nilotinib in the ENESTnd study (NCT00471497).

Methods: ENESTnd is a phase 3, randomized, open-label study comparing nilotinib 300 mg twice daily (BID), nilotinib 400 mg BID and imatinib 400 mg once daily (QD) in patients with newly-diagnosed CML. To maximize the likelihood of defining predictive and biologically relevant gene signatures, samples from a group of poor responders (BCR-ABL1 IS > 10% by 3 months of therapy) and good responders (BCR-ABL1 IS < 0.01% by 12 months of therapy) were selected across all treatment arms. Whole blood samples collected prior to study treatment initiation were available from 112 such patients from the total 846 patients enrolled in ENESTnd, and were subjected to RNA sequencing. DMR was assessed using quantitative polymerase chain reaction transcript ratios standardized to the international scale (IS) and was defined as BCR-ABL1 IS ≤ 0.01% (MR4) or ≤ 0.0032% (MR4.5). For statistical analysis, responders were defined as patients having achieved DMR by 5 years, whereas non-responders were in the trial for ≥ 5 years without achieving DMR. Five years was selected to ensure that patients on both imatinib and nilotinib arms had adequate time to reach MR4.5. The association of clinical variables with responder status (good or poor) was assessed via a multivariate logistic regression model.

Results: We correlated clinical variables (eg, Sokal risk score, TKI, age, sex) with responder status for 112 ENESTnd study patients who received 400 mg imatinib QD (n = 47), 300 mg nilotinib BID (n = 33), or 400 mg nilotinib BID (n = 32). Of the 112 patient samples, 70 were included in the analysis using MR4.5 as an endpoint, with 47 patients characterized as responders (imatinib: 16; nilotinib: 31), and 23 as non-responders (imatinib: 13; nilotinib: 10). Of the 112 samples, 42 were excluded from analysis because the patients discontinued the trial before 5 years and did not achieve MR4.5 (imatinib: 18; nilotinib: 24). Younger age (< 35 years) was associated with good response (p < 0.02) in a multivariate analysis.
We developed a predictive model of responder status by applying penalized regression to clinical variables and gene expression (13569 genes) in independent (clinical or gene expression) and combined gene and clinical models. The best performing model used patients with MR4.5 vs poor responders, with an area under the receiver operating characteristic (ROC) curve (AUC) of 0.87 (Figure; Table). Including clinical variables did not result in markedly different performance (AUC = 0.85). Significantly, both models outperformed a model that included clinical variables only (AUC = 0.65). Relaxing the definition of good responders to include patients with MR4 yielded similar results (Table).

Detailed biomarker/pathway analysis to explore the biological pathways that separate good and poor response are underway.

**Conclusions:** We present a gene expression model that distinguishes patients who achieved a DMR from those with a poor response to treatment at 5 years. The approach for sample selection optimized the chances of finding a biological and clinical signal and may be applicable to all CML patients initiating TKI therapy. This work could yield new therapeutic targets that could potentially turn a patient biologically determined to be a poor responder into a good responder who might even achieve a TFR.

![Figure: Expression-based model predicts DMR. ROC curve assessing predictive model of DMR response (using gene expression; red curve). Random model performance: dashed line.](image)

<table>
<thead>
<tr>
<th>Clinical endpoint</th>
<th>Genes (AUC)</th>
<th>Genes + Clinical (AUC)</th>
<th>Clinical (AUC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR4*</td>
<td>0.87</td>
<td>0.85</td>
<td>0.65</td>
</tr>
<tr>
<td>MR2*</td>
<td>0.84</td>
<td>0.84</td>
<td>0.69</td>
</tr>
</tbody>
</table>
666 High-Risk Additional Chromosomal Abnormalities in CML Herald Death By Blast Crisis Already at Low Blast Levels

Rüdiger Hehlmann, MD

Background. The end phase or metamorphosis is one of the remaining challenges of chronic myeloid leukemia (CML) management. Blast crisis (BC) is a late marker. Earlier diagnosis may improve outcome. The detection of additional chromosomal abnormalities (ACA) at low blast levels might allow earlier treatment when outcome is better.

Methods. We made use of 1536 Ph+CML-patients in chronic phase followed in the randomized CML study IV (Hehlmann et al, Leukemia 2017) for a median of 8.6 years. 1510 cytogenetically evaluable patients were analyzed for ACA and blast increase (Flow chart). According to impact on survival ACA were grouped into high-risk (+8; +Ph; i(17q); +17; +19 +21; 3q26; 11q23; -7; complex) and low-risk (all other). Prognosis with +8 alone was clearly better than with +8 accompanied by further abnormalities, but still worse than with low-risk ACA. +8 alone was therefore included in the high-risk group. The presence of high- and low-risk ACA was linked to 6 thresholds of blast increase (1%, 5%, 10%, 15%, 20%, and 30%) in a Cox proportional hazards model.

Results. 139 patients (9.2%) displayed ACA at any time before BC diagnosis, 88 (5.8%) had high-risk and 51 (3.4%) low-risk ACA. ACA emerged after a median of 17 (0-133) months. 79 patients developed BC. 43 (61%) of 71 cytogenetically evaluable patients with BC had high-risk ACA. 3-year survival after emergence of high-risk ACA was 48%, after emergence of low-risk ACA 92%. At low blast levels (1-15%), high-risk ACA showed an increased hazard to die (ratios: 3.66 in blood; 6.84 in marrow) compared to no ACA in contrast to low-risk ACA. This effect was not observed anymore at blast increases to 20-30% (Figure). 38 patients with high-risk ACA died, 36 with known causes of death which were almost exclusively BC (n=26, 72%) and progression-related transplantation (n=8, 22%). Only 2 patients died of CML-unrelated causes.

Conclusions. High-risk ACA herald death by BC already at low blast levels and may help to define CML end phase in a subgroup of patients at an earlier time than is possible with current blast thresholds. Cytogenetic monitoring is indicated when signs of progression surface and response to therapy is unsatisfactory. More intensive therapy may be indicated at emergence of high-risk ACA.
Flow chart. Patient flow for Cox modelling

- Total 1536
  - Patients with at least one evaluable cytogenetic analysis 1510
    - Low-risk 51
    - ACA 139
    - High-risk 88
      - 5 died
        - 3 CML related (1 BC, 2 after SCT)
        - 2 of unknown reasons
      - 38 died
        - 36 with known causes
          - 34 CML related (94%)
          - 26 BC (76.5%)
          - 8 after SCT (23.5%)
          - 2 CML-unrelated
    
- cyogenetically evaluable 71
  - ACA 45
    - High-risk 43
    - Low-risk 2
  - no ACA 26

Cox proportional hazard model

- 6 patient groups with blast levels of 1% to >30% in blood, n = 78-224
- 6 patient groups with blast levels of 1% to >30% in marrow, n = 79-1033

Figure. Hazard ratio for mortality with high- and low-risk ACA with 6 thresholds of blast increase (1-30%) in blood and marrow.