Review article

Monitoring the Response to Tyrosine Kinase Inhibitor (TKI) Treatment in Chronic Myeloid Leukemia (CML)

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Abstract. The aim of oral tyrosine kinase inhibitor (TKI) treatment in chronic myeloid leukemia (CML) is to get ideal hematological, cytogenetic, molecular responses at the critical time points. The depth of the response obtained with TKI and the time to achieve this response are both important in predicting the prognosis in patients with CML. The high efficacy of the TKI treatment of CML has prompted the need for accurate methods to monitor response at levels below the landmark of CCyR. Quantification of BCR-ABL transcripts has proven to be the most sensitive method available and has shown prognostic impact with regard to progression-free survival. European LeukemiaNet (ELN) molecular program harmonized the reporting of results according to the IS (International harmonization of Scale) in Europe. The aim of this review is to outline monitoring the response to optimal TKI treatment based on the ELN CML 2013 recommendations from the clinical point of view as a physician. Careful cytogenetic and molecular monitoring could help to select the most convenient TKI drug and to optimize TKI treatment. Excessive monitoring may have an economical cost, but failure to optimize TKI treatment may result in CML disease acceleration and death.

Introduction. Current standard therapy for chronic phase (CP-) Ph+ Chronic myeloid leukemia (CML) is the chronic oral administration of tyrosine kinase inhibitor (TKI) drug. European LeukemiaNet (ELN) 2013 recommendations provided clear, practical suggestions for the physicians dealing with CML management, based on the best available evidence about the TKI drugs, without disregarding clinical realities and expectations. The aim of this review is to outline monitoring the response to optimal TKI treatment based on the ELN CML 2013 recommendations from the clinical point of view as a physician.

Based on the true ELN philosophy, the cost of monitoring is much lower than the cost of the TKI drugs.

Careful cytogenetic and molecular monitoring could help selecting the most convenient TKI drug and to optimize TKI treatment. Excessive monitoring may have an economical cost, but failure to optimize TKI treatment may result in CML disease acceleration and death. Insufficient diagnostic/therapeutic clinical
intervention during the management of CML disease course with TKI drugs can cause accelerated phase (AP) or blastic crisis (BC). The survival after the progression into AP/BC is still significantly shorter even in the powerful TKI era.\textsuperscript{2}

Diagnostic Tools and Surrogate Markers for the Monitoring the Response to TKI in CML. Ph+ CML disease burden should be monitored during the TKI treatment.\textsuperscript{3} Hematologic response (HR) is measured by the evaluation of complete blood counts (CBC), white blood cell differential (WBC), and assessment of the spleen size. The definition of the hematologic, cytogenetic and molecular responses are depicted in Table 1. Cytogenetic response (CyR) is detected via the chromosome banding analysis of the bone marrow cell metaphases. The principle of the molecular response (MR) depends upon the measurement of the BCR-ABL transcript levels relative to a control gene. After one year of TKI treatment in CML, complete (C) HR can be obtained in about 98%, CCyR in 57-88\%, and major (M)MR in 18-58\% of the patients.\textsuperscript{1,4,6}

Optimal Cytogenetic and Molecular Monitoring in CML Based on ELN 2013 Recommendations. The responses to TKI in CML can be assessed either with molecular tests alone or with cytogenetic tests alone, depending on the local laboratory facilities.\textsuperscript{1,7-14} However, both cytogenetic and molecular tests are recommended, until a CCyR and an MMR are achieved. Then quantitative molecular tests from the peripheral blood samples alone may be sufficient.\textsuperscript{1}

The molecular ELN CML 2013 recommendations are; quantitative RT-PCR of blood cells every 3 months, until the MMR is achieved and confirmed, and then RT-PCR every 3 to 6 months. The molecular results must be expressed by the IS (International harmonization of Scale).\textsuperscript{1}

The cytogenetic ELN CML 2013 recommendations are; chromosome banding analysis (CBA) of marrow cell metaphases at 3 and 6 months, then every 6 months until the CCyR is achieved. CBA of the bone marrow cells should be repeated at least every 12 months only if the molecular response cannot be measured. FISH of the blood cells can substitute for CBA only if bone marrow cells cannot be obtained, and only for the definition of CCyR.\textsuperscript{1}

Mutational analysis is recommended in case of progression, failure and warning based on the ELN CML 2013\textsuperscript{1,15} recommendations. In case of failure, warning, and of development of myelodysplastic features (unexpected leukopenia, thrombocytopenia, or anemia), CBA of the bone marrow cell metaphases is recommended.\textsuperscript{1}

Monitoring TKI Response at the Critical Time Points in CML Based on ELN 2013 Recommendations. At the diagnosis of CML; CBA of the marrow cell metaphases, FISH in case of Ph negativity, to identify variant, cryptic translocations and qualitative PCR (identification of transcript type) are required.\textsuperscript{1}

During the treatment of CML; Quantitative, real-time PCR (RQ-PCR) for the determination of

Table 1. The definition of the hematologic, cytogenetic and molecular responses in CML.

| Type of Response | Definition |
|------------------|------------|
| CHR              | Complete Hematologic Response | Normal differential, WBC & platelets ≤ ULN |
| MCR              | Major cytogenetic Response | 0-35% Ph+marrow metaphases |
| CCyR             | Complete Cytogenetic Response | 0% Ph+marrow metaphases |
| MMR              | Major Molecular Response | BCR-ABL/ABL ≤ 0.1% (International Scale) |
| MR\textsuperscript{4.0} | BCR-ABL/ABL ≤ 0.001% (IS) “4-log reduction” |
| MR\textsuperscript{4.5} | BCR-ABL/ABL ≤ 0.003% (IS) “4.5-log reduction” |
| CMR              | Complete Molecular Response | Undetectable BCR-ABL (test of sensitivity ≥ 4.5 logs) |
BCR/ABL\(^1\) transcripts level on the international scale, to be performed every 3 months until an MMR has been achieved, then every 3 to 6 months and/or CBA of the bone marrow cell metaphases (at least 20 banded metaphases), to be performed at 3, 6 and 12 months until a CCyR has been achieved, then every 12 months. Once a CCyR is achieved, FISH on blood cells can be used. If an adequate molecular monitoring can be assured, cytogenetics can be spared.\(^1\)

In the case of failure or progression of CML; RQ-PCR, mutational analysis, and CBA of the bone marrow cell metaphases and immunophenotyping in blastic phase are required.

When a ‘Warning’ sign appeared during the TKI administration in CML based on ELN 2013; Molecular and cytogenetic tests to be performed more frequently. CBA of the bone marrow cell metaphases recommended in case of myelodysplasia or complex karyotypic abnormalities (CCA)/Ph+ with chromosome 7 involvement.\(^1\)

**Ideal Response Level to the TKI Treatment Detectable During the Long-Term Monitoring in CML.** The ideal responses to the TKI treatment detectable during the long-term monitoring of CML are depicted in Table 2. Inability to detect ELN-warnings in a CML patient receiving a given TKI, resulting in drug failure and/or disease progression can cause damage to the patient.\(^\) Proper therapeutic interventions in case of primary and secondary failures during the TKI treatments are described in the ELN 2013 recommendations.\(^1\)

**Clinical Significance of the Ideal Response Level to the TKI Treatment Detectable During the Long-Term Monitoring in CML.** The aim of TKI treatment in CML is to get ideal hematological, cytogenetic, molecular responses in the critical time-points (at the 3rd month, at the 6th month, after one year, and thereafter) as depicted in Table 1. The depth of the response obtained with TKI and time to achieve this response are important for the prediction of prognosis in the patient with CML.\(^\) Clinical significances of the ideal response level to the TKI treatment detectable during the long-term monitoring in CML are indicated below.

**CHR:** complete hematological response is defined as normal CBC, normal peripheral blood smear and normal spleen in the physical examination.\(^\) Absence of CHR at any stage during the CML disease course is a clear sign of disease progression. Proper therapeutic intervention in the absence of CHR during the TKI treatments is described in the ELN 2013 recommendations.\(^1\)

**CCyR:** complete cytogenetic response is defined as the absence of Ph+ chromosome in the CBA of the bone marrow cells in at least 20 banded metaphases. CCyR is the golden standard during the TKI treatment and must be obtained within the first year (ideally at the six months of TKI regimen) and should be maintained during the long-term management of CML. CCyR is a significant barrier against the CML disease progression. CCyR can be achieved in about 57-88% of the patients with CML in the TKI era. Absence of CCyR after one year of CML disease course is a great danger for disease progression. Proper therapeutic intervention in the absence of CCyR during the TKI treatments is described in the ELN 2013 recommendations.\(^1\)

**MMR:** major molecular response is defined as BCR-ABL ≤ 0.1% in the quantitative RT-PCR of blood cells. MMR is a safe haven during the TKI treatment and must be obtained within the 18 months (ideally at the 12th months of TKI regimen) and should be maintained during the long-term management of CML. MMR is a very significant barrier against the CML disease progression. MMR can be achieved in about 18-58% of the patients with CML in the TKI era. Absence of MMR after 18 months of CML disease course is a danger for disease progression. Proper therapeutic intervention in the absence of MMR during the TKI treatments is described in the ELN 2013 recommendations.\(^1\)

| Table 2: The ideal responses to the tyrosine kinase inhibitor (TKI) treatment detectable during the long-term monitoring of chronic myeloid leukemia (CML). |
|---------------------------------------------------------------|
| **Ideal response at the 3rd month of TKI** | **Ideal response at the 6th month of TKI** | **Ideal response at the 12th month of TKI** | **Ideal response after one year of TKI and thereafter** |
| **Hematological monitoring** | CHR | CHR | CHR | CHR |
| **Cytogenetic monitoring** | MCyR | CCyR | CCyR | CCyR |
| **Molecular monitoring** | BCR-ABL/ABL below 10% | BCR-ABL/ABL below 1% | MMR | better than MMR; MR4, MR4.5, MR5 |

CHR: complete hematological response. MCyR: major cytogenetic response. CCyR: complete cytogenetic response. MMR: major molecular response.
**EMR**: early molecular response is defined as BCR-ABL/ABL ≤ 10% cut-off in the quantitative RT-PCR of blood cells.\(^{18,19}\) EMR (ideally at the 3rd month of TKI treatment) can predict long-term prognosis during the TKI treatment and must be reached within the first 6 months during the management of CML.\(^{3,16,20}\) EMR is a prognostic sign for CML disease course. EMR can be achieved in about 91% of the patients with CML receiving nilotinib and 67% receiving imatinib in the ENESTnd trial.\(^{21}\) Absence of EMR after 6 months of CML disease course represents an aggressive disease course in the long-term for instance after 5 years. Proper therapeutic intervention in the absence of EMR during the TKI treatments is described in the ELN 2013 recommendations.\(^1\)

TFR (treatment-free remission) is the discontinuation of TKI in the superior-TKI responder patient with CML. The deeper molecular responses (MR4, MR4.5, MR5) detected during at least two years of monitoring in CML are candidates for TFR. MR4 can be achieved by a BCR-ABL expression < 0.01%, MR4.5 by <0.0032% BCR-ABL\(^{15}\), and MR5 by <0.001% BCR-ABL\(^{15}\). Young and low prognostic risk CML patients are candidates of first line 2nd generation TKIs with the aim of drug discontinuation in their future life.\(^6\)

Mutational analyses shall only be performed in patients with suboptimal responses, warnings, and failures in CML cases subject to the alterations in the treatment strategies.\(^1\) Mutations detected during the TKI therapy may be resulted in drug switches based on the nature of the mutation. T315I, Y253K, E255K, E255V, F359V, F359C, are the mutations poorly sensitive to nilotinib; whereas T315I, T315A, F317L, F317C, V299L are the mutations poorly sensitive to dasatinib. T315I is a unique mutation making the CML patient irresponsive to all available TKIs but ponatinib and allografting.\(^{22,29}\)

Patients with advanced phase (AP/BC) CML are currently treated with the most powerful TKI\(^{30}\) available (dasatinib\(^{31}\) or ponatinib\(^{32}\)) and multi-agent chemotherapy before allografting. Monitoring of those patients is also problematic. Durable hematological, cytogenetic, molecular responses are hard to be obtained in the CML patients with advanced phase (AP/BC) disease. Although durable hematologic, cytogenetic and molecular responses can be hardly obtained in AP and particularly in BP patients, the definition of the responses should be the same as for CP patients. Proper therapeutic interventions in advanced phase CML are described elsewhere.\(^{1,2}\)

**Practical Problems in the Long-Term Monitoring of TKI Treatment in CML**. CHR, early CCyR, faster MMR, and the deeper, durable molecular responses (MR4, MR4.5, MR5) are the ultimate goals of the TKI-receiving patients with CML. Critical evaluations of the CML patients to hit those targets shall be made at the baseline, and at the 3rd, 6th, 12th month, and thereafter the TKI administration. There are some practical and technique-related problems during the hematological, cytogenetic, molecular monitoring of TKI treatment in CML. Clinical significances of those incidences during the long-term monitoring in CML are indicated below:

Hydroxyurea treatment, especially in sustained high doses, before the initiation of TKI regimen, could obscure the evaluation of CHR and baseline CML disease risk profile of the patient. Before the TKI decision, the baseline assessments of the de novo CML patient shall include exact medical diagnosis of CML, basic laboratory evaluation covering CBC and peripheral blood smear (PBS), bone marrow cytology, conventional cytogenetics and/or FISH analyses for Ph+ chromosome, and qualitative molecular analyses for the BCR-ABL.\(^1\) Tumor load and disease phase should be defined. Newly diagnosed CP-CML patients should be stratified based on the Sokal, Euro/Hasford and EUTOS CML prognostic scoring systems.\(^{33}\) Hydroxyurea can affect CBC, PBS, spleen size, bone marrow cellularity, the quality of metaphases, and essential parameters of the Sokal, Euro/Hasford and EUTOS CML prognostic scoring systems. Therefore, baseline CML disease risk profile of the patient shall be obtained before the hydroxyurea and/or TKI were administered to the patient.

The estimated ratio of BCR-ABL/ABL is highly technique-dependant. Many laboratories in the world are not yet qualified for the international harmonization of scale (IS). Standardization of BCR-ABL quantification in Europe have been performed by European LeukemiaNet (ELN) and the European Treatment, and Outcome Study (EUTOS).\(^{34}\) The high efficacy of the TKI treatment of CML has prompted the need for accurate methods to monitor response at levels below the landmark of CCyR. Quantification of BCR-ABL transcripts has proven to be the most sensitive method available and has shown prognostic impact with regard to progression-free survival. The variations in the methods used to quantify BCR-ABL made it difficult to compare results between laboratories. ELN program harmonized the reporting of results according to the IS in Europe. The ELN recommendations for the propagation of the IS by national or regional laboratory networks.\(^{34}\) The 2012 status of the BCR-ABL standardization within 64 participating laboratories in 28 countries including the Mediterranean land is depicted in Figure 1.

Regarding the EMR, the challenges for the widespread routine use of the 10% BCR-ABL
transcript cut-off at the 3rd month of TKI are present. High ratio values on IS scale, housekeeping control gene problem, variations in the samples, delays in the exact molecular assessment time after TKI and early unexpected variation kinetics of response in individual CML patients complicate the interpretation of the 10% BCR-ABL transcript cut-off at the 3rd month of TKI. Likewise, the tumor burden at diagnosis, prognostic scoring, gene profile, cytoreduction before TKI, treatment adherence, and numerous confounding effects may obscure the real-life decision at the 3rd month of TKI outside the clinical trials. Nevertheless, obtaining faster, deeper and durable molecular responses particularly MMR are essential for the patient with CML in the TKI era. Proper therapeutic interventions based on the molecular responses are described in the ELN 2013 recommendations.\(^1\)

The cytogenetic analyses also have technique-dependent problems. Obtaining the CBA of the bone marrow cell metaphases at 3 and 6 months, then every 6 months until the CCyR\(^1\) could not be possible in all cases of CML under TKI. Invasive nature of the bone marrow aspiration/biopsy could represent another clinical problem. FISH of the blood cells can substitute CBA if bone marrow cells cannot be obtained for the definition of CCyR. The standardization about the sensitivity level of FISH has improved. Nevertheless, obtaining earlier and stable cytogenetic responses particularly CCyR are essential for the patient with CML in the TKI era. Proper therapeutic interventions based on the cytogenetic responses are described in the ELN 2013 recommendations.\(^1\)

CML treatment may be modelled on the individual disease and patients characteristics (risk, molecular profile, age, co-morbidities, aggressive clinical course, etc.). Therefore, the CML monitoring strategy to detect the response to TKI may also be varied and tailored on an individual basis. Drug tolerability, patient compliance of TKI, physician adherence to TKI, and off-target TKI complications should always be monitored during the CML treatment. Otherwise, late, off-target complications of TKI (lung toxicity,\(^35\) cardiac toxicity,\(^36,37\) metabolic syndrome\(^21\), bone toxicity,\(^38\) arterial and venous occlusive events,\(^39\) pancreas toxicity\(^1\), and others) may limit the benefits of the given TKI. Proper therapeutic interventions based on the therapeutic monitoring of the CML patients and TKI drugs are described in the ELN 2013 recommendations.\(^1\)
Future Perspectives in the Monitoring of TKI Treatment in CML. Novel recent investigations for the de novo CML patients have searched the validity of gene expression profiling, genetic polymorphisms, next generation genomics, multi-drug resistance genes (MDR, OCT1), fusion transcripts and pre-existing BCR-ABL kinase domain mutations. The cessation of the TKI therapy with the aim of cure, stem cell depletion, stem cell exhaustion, immunological control of the disease will be the future therapeutic tools of CML. The improvements in the international harmonization of scale about the molecular monitoring would be very important in the TFR stage of CML with the intention to cure the disease.

References:

1. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood 2013;122:872-84. http://dx.doi.org/10.1182/blood-2013-05-501569 PMid:23803709

2. Hehlmann R. How I treat CML blast crisis. Blood 2012;120:737-47. http://dx.doi.org/10.1182/blood-2012-03-380147 PMid:22653972

3. Guilhot F, Roy L, Tomowiak C. Current treatment strategies in chronic myeloid leukemia. Current opinion in hematology 2012;19:102-9. http://dx.doi.org/10.1097/MOH.0b013e32834ff610 PMid:2227529

4. Baccarani M, Castagnetti F, Porkka K, et al. A prospective study of imatinib 400 mg vs 800 mg frontline in high risk Ph plus chronic myeloid leukemia (CML) patients. Blood 2007;110:16A.

5. Baccarani M, Castagnetti F, Simonsson B, et al. Cytogenetic and Molecular Response to Imatinib in High Risk (Sokal) Chronic Myeloid Leukemia (CML): Results of A European Leukemianet Prospective Study Comparing 400 Mg and 800 Mg Front-Line. Blood 2008;112:75-6.

6. Baccarani M, Cortes J, Pane F, et al. Chronic myeloid leukemia: an update of concepts and management recommendations of European LeukemiaNet. Journal of Clinical Oncology 2009;27:6041-51. http://dx.doi.org/10.1200/JCO.2009.25.0779 PMid:19884523

7. Brecchia M, Alimena G. The significance of early, major and stable molecular responses in chronic myeloid leukemia in the imatinib era. Critical reviews in oncology/hematology 2011;79:135-43. http://dx.doi.org/10.1016/j.critrevonc.2010.07.003 PMid:20685131

8. Castagnetti F, Gugliotta G, Brecchia M, et al. The BCR-ABL Transcript Levels At 3 and 6 Months Predict the Long-Term Outcome of Chronic Myeloid Leukemia Patients Treated Frontline with Imatinib Mesylate: A Gimema CML WP Analysis. ASH Annual Meeting Abstracts 2012;120:1678.

9. Cortes JE, Pasquini R, Kantarjian HM, et al. First-Line Treatment and Management of Chronic Myeloid Leukemia (CML) in Clinical Practice: Update of > 1800 Patients (Pts) in the WORLD CML Registry. ASH Annual Meeting Abstracts 2012;120:3750.

10. Faussel C. Targeted chronic myeloid leukemia therapy: Seeking a cure. American journal of health-system pharmacy 2007;64:S9-15. http://dx.doi.org/10.2146/ajhp070482 PMid:18059932

11. Gugliotta G, Castagnetti F, Palandrì F, et al. Cytogenetic and Molecular Responses At 3 Months Are Associated With A Better Outcome in Early Chronic Phase (ECP) Chronic Myeloid Leukemia (CML) Patients Treated with Nilotinib. ASH Annual Meeting Abstracts 2012;120:2797.

12. Merillon LA, Sandoval-Sanchez C, Delgado-Lopez N, et al. Molecular Status Evaluation in a Standardized Laboratory in a Cohort of Patients with Chronic Myeloid Leukemia At the Instituto Mexicano Del Seguro Social (IMSS). ASH Annual Meeting Abstracts 2012;120:4452.

13. Pagnano KB, Ribeiro BF, Miranda ECM, et al. Early Assessment of Molecular Response in Chronic Myeloid Leukemia Patients On Dasatinib After Imatinib Failure Identify Patients with Poor Cytogenetic and Molecular Responses. ASH Annual Meeting Abstracts 2012;120:3787.

14. Rousselot P, Mollica L, Etienne G, et al. Pharmacologic Monitoring of Dasatinib As First Line Therapy in Newly Diagnosed Chronic Phase Chronic Myelogenous Leukemia (CML-CP) Identifies Patients At Higher Risk of Pleural Effusion: A Sub-Analysis of the OPTIM-Dasatinib Trial. ASH Annual Meeting Abstracts 2012;120:3770.

15. White DL, Radich J, Soverini S, et al. Chronic phase chronic myeloid leukemia patients with low OCT-1 activity randomized to high-dose imatinib achieve better responses and have lower failure rates than those randomized to standard-dose imatinib. Haematologica 2012;97:907-14. http://dx.doi.org/10.3324/haematol.2011.056457 PMid:22207690 PMcid:PMC336658

16. Haznedaroglu IC. Current concerns of undertreatment and overtreatment in chronic myeloid leukemia based on European LeukemiaNet 2013 recommendations. Expert opinion on pharmacotherapy 2013;14:2005-10. http://dx.doi.org/10.1517/14656566.2013.833185 PMid:23984805

17. Hayran M, Koca E, Haznedaroglu IC, et al. Predicting chronic leukaemias from assessment of complete peripheral blood counts. The Journal of international medical research 2006;34:640-7. http://dx.doi.org/10.1177/14732300060034000609 PMid:17294996

18. Haznedaroglu IC: Key Decision Just After 3 Months Following TKI Initiation in CML: Better and not Difficult. Uhod-Uluslararasi Hematoloji-Onkoloji Dergisi 2013;23:1-2.

19. Hanfstein B, Müller M, Hehlmann R, et al. Early molecular and cytogenetic response is predictive for long-term progression-free and overall survival in chronic myeloid leukemia (CML). Leukemia 2012;26:2096-102. http://dx.doi.org/10.1038/leu.2012.85 PMid:22446502

20. Cortes J, Kantarjian H, How I treat newly diagnosed chronic phase CML. Blood 2012;120:1390-7. http://dx.doi.org/10.1182/blood-2012-03-378919 PMid:22613793

21. Saglio G, Kim DW, Issaragrisil S, et al. Nilotinib versus imatinib for newly diagnosed chronic myeloid leukemia. The New England journal of medicine 2010;362:2251-9. http://dx.doi.org/10.1056/NEJMoa0912614 PMid:20525993

22. Aggoune D, Sorel N, Bonnet ML, Chomel J-C, Turhan AG. A Niche-Based Cell Mutagenesis Assay Identifies ABL-Kinase Mutations Appearing in BCR-ABL T315I-Mutated Leukemic Cells Treated with Ponatinib. ASH Annual Meeting Abstracts 2012;120:2436.

23. Cillonzi D, Bracco E, Carturan S, et al. Design and Application of a Novel PNA Probe for the Detection At a Single Cell Level of BCR-ABL T315I Mutation in Chronic Myeloid Leukemia Patients. ASH Annual Meeting Abstracts 2012;120:3732.

24. Kim S-H, Choi SY, Lee S-E, et al. Dynamics and Characteristics of BCR-ABL1 Multiple Mutations in Tyrosine Kinase Inhibitor Resistant CML. ASH Annual Meeting Abstracts 2012;120:1677.

25. Li J, Liu W, Yan Z, et al. Next-Generation Sequencing of the BCR-ABL1 Kinase Domain and Neighboring Domains Associated with Therapy Resistance. ASH Annual Meeting Abstracts 2012;120:2549.

26. Maier J, Schubert K, Cross M, et al. Low Level BCR-ABL Mutations Below the Detection Limit of Current Standard Screening Techniques Occur Predominantly in the CD34+ Progenitor Cell Compartment in Chronic Phase CML Patients At Diagnosis. A Substudy of the ENEST1st Trial. ASH Annual Meeting Abstracts 2012;120:1671.

27. Soverini S, De Benedittis C, Polakova KM, et al. Dissecting the Complexity of Philadelphia-Positive Mutated Populations by Ultra-Deep Sequencing of the Bcr-Abl Kinase Domain: Biological and Clinical Implications. ASH Annual Meeting Abstracts 2012;120:692.

28. Soverini S, De Benedittis C, Polakova KM, et al. Ultra-Deep Sequencing of the Bcr-Abl Kinase Domain Allows Earlier Detection and More Accurate Characterization of Resistant cr...
Subclones in Philadelphia-Positive Acute Lymphoblastic Leukemia Patients Receiving Tyrosine Kinase Inhibitor-Based Therapies. ASH Annual Meeting Abstracts 2012;120:284.

29. Zhang WW, Cortes JE, Yao H, et al. Predictors of primary imatinib resistance in chronic myelogenous leukemia are distinct from those in secondary imatinib resistance. Journal of clinical oncology 2009;27:3642-9. http://dx.doi.org/10.1200/JCO.2008.19.4076 PMid:19506164 PMCID:PMC2799062

30. Beyazit Y, Kekilli M, Haznedaroglu IC. Second-generation BCR-ABL kinase inhibitors in CML. The New England journal of medicine 2010;363:1673. PMid:20973146

31. Saydam G, Haznedaroglu IC, Temiz Y, et al. Retrospective evaluation of patients treated with dasatinib for Philadelphia positive leukemias: Turkish experience of 16 months. UHOD 2009;19:195-204. http://dx.doi.org/10.4999/uhod.2009.19.09137

32. Cortes JE, Kim D-W, Pinilla-Ibarz J, et al. A Pivotal Phase 2 Trial of Ponatinib in Patients with Chronic Myeloid Leukemia (CML) and Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia (Ph+ALL) Resistant or Intolerant to Dasatinib or Nilotinib, or with the T315I BCR-ABL Mutation: 12-Month Follow-up of the PACE Trial. ASH Annual Meeting Abstracts 2012;120:163.

33. Uz B, Buyukasik Y, Atay H, et al. EUTOS CML prognostic scoring system predicts ELN-based 'event-free survival' better than Euro/Hasford and Sokal systems in CML patients receiving frontline imatinib mesylate. Hematology 2013;18:247-252. http://dx.doi.org/10.1179/1607845412Y.0000000071 PMid:23540886

34. Muller MC, Cross NC, Erben P, et al. Harmonization of molecular monitoring of CML therapy in Europe. Leukemia 2009;23:1957-63. http://dx.doi.org/10.1038/leu.2009.168 PMid:19710700

35. Aksu S, Bektas O, Uz B, et al. Effective Molecular Monitoring and the Proper Management of Pleural Effusion During the First-Line Dasatinib Administration in CML. Uhod-Uluslararası Hematoloji-Onkoloji Dergisi 2012;22:1-7.

36. Sayitoglu M, Haznedaroglu IC, Hatimaz O, et al. Effects of imatinib mesylate on renin-angiotensin system (RAS) activity during the clinical course of chronic myeloid leukaemia. The Journal of international medical research 2009;37:1018-28. http://dx.doi.org/10.1177/147323000903700406 PMid:19761684

37. Uz B, Tatonyan SC, Sayitoglu M, et al. Local hematopoietic renin-angiotensin system in myeloid versus lymphoid hematological neoplastic disorders. Journal of the renin-angiotensin-aldosterone system : JRAAS 2013 (in press). http://dx.doi.org/10.1177/1470320312464672 PMid:23132846

38. Tauer JT, Hofbauer LC, Erben RG, Suttorp M. Skeletal Effects of the Tyrosine Kinase Inhibitors Imatinib, Dasatinib, and Bosutinib in Young Rats. ASH Annual Meeting Abstracts 2012;120:4429.

39. Schwarz M, Kim TD, Mirault T, et al. Elevated Risk of Peripheral Artery Occlusive Disease (PAOD) in Nilotinib Treated Chronic Phase Chronic Myeloid Leukemia (CML) Patients Assessed by Ankle-Brachial-Index (ABI) and Duplex Ultrasonography. ASH Annual Meeting Abstracts 2012;120:914.

40. Horn M, Glauhe I, Muller MC, et al. Model-based decision rules reduce the risk of molecular relapse after cessation of tyrosine kinase inhibitor therapy in chronic myeloid leukemia. Blood 2013;121:378-384. http://dx.doi.org/10.1182/blood-2012-07-441956 PMid:23175686