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Brief report

SNP array analysis of tyrosine kinase inhibitor-resistant chronic myeloid leukemia identifies heterogeneous secondary genomic alterations

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To elucidate whether tyrosine kinase inhibitor (TKI) resistance in chronic myeloid leukemia is associated with characteristic genomic alterations, we analyzed DNA samples from 45 TKI-resistant chronic myeloid leukemia patients with 250K single nucleotide polymorphism arrays. From 20 patients, matched serial samples of pretreatment and TKI resistance time points were available. Eleven of the 45 TKI-resistant patients had mutations of *BCR-ABL1*, including 2 T315I mutations. Besides known TKI resistance-associated genomic lesions, such as duplication of the *BCR-ABL1* gene (n = 8) and trisomy 8 (n = 3), recurrent submicroscopic alterations, including acquired uniparental disomy, were detectable on chromosomes 1, 8, 9, 17, 19, and 22. On chromosome 22, newly acquired and recurrent deletions of the IGLC1 locus were detected in 3 patients, who had previously presented with lymphoid or myeloid blast crisis. This may support a hypothesis of TKI-induced selection of subclones differentiating into immature B-cell progenitors as a mechanism of disease progression and evasion of TKI sensitivity. (Blood. 2010;115:1049-1053)

Introduction

Chronic myeloid leukemia (CML) patients can develop secondary resistance in the course of treatment with tyrosine kinase inhibitors (TKIs), such as imatinib, nilotinib, or dasatinib.[1,2] The main known causes for resistance are mutations or overexpression of the BCR-ABL1 fusion protein, reduced bioavailability of the drugs, and activation of compensatory molecular pathways.[2,3] CML cells exhibit increased genomic instability, which could lead to genomic lesions harboring additional mechanisms of resistance. Use of high-density single nucleotide polymorphism (SNP) arrays in combination with a new computational algorithm termed "molecular allelokaryotyping" allows robust and detailed detection of cryptic micro-deletions, micro-amplifications, and loss of heterozygosity (LOH), including acquired uniparental disomy.[4,5] We performed a genomic DNA profiling of 45 TKI-resistant CML patients with 250K SNP arrays to elucidate genomic alterations, which could be associated with TKI resistance.

Methods

Patients and DNA samples

We studied 45 cases of TKI-resistant CML. From 25 patients, DNA was extracted upon development of clinical resistance to imatinib, dasatinib, or nilotinib. From 20 patients, sequential DNA samples were available for comparison between diagnosis and development of resistance against at least 1 of the aforementioned TKIs. All patients were confirmed positive for *BCR-ABL1* by polymerase chain reaction (PCR) and fluorescence in situ hybridization. Eleven resistant patient samples had *BCR-ABL1* mutations, including 2 T315I mutations. The anonymized DNA samples were obtained from patients referred to the Munich Leukemia Laboratory, Munich, Germany. Genomic DNA was isolated from mononuclear cells from bone marrow aspirates or leukemic peripheral blood. The acquisition and analysis of DNA samples from the patients were with approval of the ethical committee of all participating institutions. A detailed summary of patient information is given in supplemental Table 1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

High-density SNP array analysis

High-quality genomic DNA was processed according to the genomic mapping 250K NspI protocol and hybridized to 250K NspI SNP arrays according to the manufacturer's instructions (Affymetrix). Data analysis of deletions, amplifications, and uniparental disomy was carried out using the CNAG software with nonmatched references as previously

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Table 1. Deletions found in the TKI-resistant CML samples

ID	Chromosome	Starting position	Ending position	Length, Mb	Candidate genes	Treatment	Disease status
2	1p36	8206962	9061724	0.85	*RERE, ENO-1*	Imatinib, DA, nilotinib, dasatinib	Myeloid blast crisis
40-R*	2p14	67344057	67586748	0.24	*ETAA1*	Imatinib	Myeloid blast crisis
40-R*	2p14	67726052	67862441	0.13	No gene	Imatinib	Myeloid blast crisis
40-R*	3q26	182773194	183414855	0.64	*SOX2*	Imatinib	Myeloid blast crisis
45-R*	3q13	107395968	113108377	5.71		Imatinib	Unknown
34	6p22	21716958	22106421	0.39	*FLJ22536*	HU, ICT, imatinib, dasatinib	Lymphoid blast crisis
45-R*	6p12	47456409	54815576	7.35		Imatinib	Unknown
40-R*	8p	180568	28696690	28.51	*INTS9*	Imatinib	Myeloid blast crisis
45-R	8q12	56513192	56568360	0.05	*SBF1B1, homo del*	Imatinib	Unknown
34	9p	30910	130700428	130.70	*Del der Chromosome 9*	HU, ICT, imatinib, dasatinib	Lymphoid blast crisis
12	9q34	128779763	130659964	1.88	*ABL1-PHYHD1*	Imatinib	Unknown
32-R	9q34	128779763	130643119	1.86	*ABL1-PHYHD1*	Imatinib	CML in hematologic remission
41-R	9q34	128779763	130760738	1.98	*ABL1-PHYHD1*	Imatinib	Unknown
44-R	9q34	129888844	130775671	0.88	*ABL1-GPR107*	Imatinib	CML in hematologic remission
43-R	9q34	130889873	131275779	0.38	*LAMC3, AIF1L, NUP214* and more	Imatinib	Unknown
40-R8	9q33	117671985	119985986	2.31	*DBC1*	Imatinib	Myeloid blast crisis
33-R	10q21	67879503	67935343	0.06	*CTNNA3*	DA, imatinib	Myeloid blast crisis
45-R*	11q	89538451	92629460	3.09		Imatinib	Unknown
34	12p12	14971302	17224159	2.25	*ERP27, RERG, PTPRO* + other genes	HU, ICT, imatinib, dasatinib	Lymphoid blast crisis
34	12p12	25334604	25429100	0.09	No gene	HU, ICT, imatinib, dasatinib	Lymphoid blast crisis
40-R*	12p11	28549306	28659885	0.11	*CCDC91*	Imatinib	Myeloid blast crisis
35	14q31	85977359	86198515	0.22	No gene	HU + IFN, imatinib	CML
34	15q15	38796960	41889601	3.09	*RAD51-MFAP1*	HU, ICT, imatinib, dasatinib	Lymphoid blast crisis
40-R*	16q	45065445	88690776	43.62		Imatinib	Myeloid blast crisis
27	17p	18901	22029237	22.01	*p53*	Imatinib	Unknown
40-R*	17p	18901	21346948	21.32	*p53*	Imatinib	Myeloid blast crisis
46-R*	17p	18901	18857962	18.83	*p53*	Unknown	CML
34	17q12	32663038	33785091	1.12	*ACACA-SOCS7*	HU, ICT, imatinib, dasatinib	Lymphoid blast crisis
2	22q11	20724157	20832986	0.11	*IGLC1*	Imatinib, DA, nilotinib, dasatinib	Myeloid blast crisis
22-R*	22q11	20724157	20832986	0.11	*IGLC1*	Imatinib	Lymphoid blast crisis
34	22q11	20685204	20859240	0.17	*IGLC1*	HU, ICT, imatinib, dasatinib	Lymphoid blast crisis
12	22q11	21970272	23413582	1.44	*BCR-GGT1*	Imatinib	Unknown
41-R	22q11	21947838	23157101	1.20	*BCR, ADORA2A*	Imatinib	Unknown
44-R	22q11	21939318	27992558	6.05	*BCR, CHEK2*	Imatinib	CML in hematologic remission
32-R	22q11	22353181	23209777	0.79	*VPREB3*	Imatinib	CML in hematologic remission
43-R	22q11	22860184	23556124	0.69	*CABIN1-PIWIL3*	Imatinib	Unknown

Copy number alterations (CNAs) in TKI-resistant samples without a matched diagnostic sample are shown with plain numbers in the column "ID." CNAs in TKI-resistant samples with a matched diagnostic sample with the CNAs being present in both the diagnostic sample and the resistant sample are shown with numbers + "R" in the column "ID." CNAs which were newly acquired in matched resistant samples compared to the diagnostic samples are shown with numbers + "R*" in the column "ID."

ID indicates patient ID; Starting position, start position of genomic lesion; Ending position, end position of genomic lesion; HU, hydroxyurea; DA, daunorubicin plus cytarabine; ICT, induction chemotherapy; and IFN, interferon-α.

described.[5,6] The SNP array data are publicly available at the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/projects/geo/) under accession number GSE18964.

Mutation analysis and validation of SNP array results

Mutation analysis of candidate genes was carried out by standard genomic PCR of all exons and subsequent direct sequencing of the amplified and purified PCR products with the BigDye Terminator, Version 3.1 Cycle Sequencing Kit (Applied Biosystems). Validation of copy number results and uniparental disomy (UPD) were carried out as previously described,[7] and results are shown in supplemental Figures 1 and 2.

Results and discussion

After exclusion of genomic copy number polymorphisms by comparison of the data with recorded copy number polymorphisms

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Table 2. Duplications present in TKI-resistant CML samples

ID	Chromosome	Starting position	Ending position	Length, Mb	Candidate genes	Treatment	Disease status
27	2p21	42500507	43164500	0.66	*KCNG3, MTA3, OXER1, HAAO*	Imatinib	Unknown
16	2q36	227668515	227890738	0.22	*RHBDD1, COL4A4, COL4A3*	HU, imatinib	CML
25	2q37	231049480	231135251	0.09	*SP100*	HU + IFN, imatinib	Unknown
11	8p-q	180568	146263538	146.1	Trisomy 8	Imatinib	CML in hematologic remission
33-R	8p-q	180568	146263538	146.1	Trisomy 8	DA, imatinib	Myeloid blast crisis
42-R*	8p-q	180568	146263538	146.1	Trisomy 8	HU, imatinib	CML in acceleration
38-R	8q24	138371297	139210288	0.84	Hypothetical protein *FLJ45872*	HU, imatinib	CML in hematologic remission
45-R	8q11	53916301	146263538	92.34		Imatinib	Unknown
27	9q34	130686824	138303776	7.62	Duplication *ABL1*	Imatinib	Unknown
16	9q34	130686824	138303776	7.62	Duplication *ABL1*	HU, imatinib	CML
24	9q34	130641293	138303776	7.66	Duplication *ABL1*	Imatinib	Unknown
5	9q34	130737915	138303776	7.57	Duplication *ABL1*	Imatinib	CML
33-R*	9q34	130737915	138303776	7.57	Duplication *ABL1*	DA, imatinib	Myeloid blast crisis
40-R*	9q34	130659964	138303776	7.64	Duplication *ABL1*	Imatinib	Myeloid blast crisis
41-R	10p11	35,083,411	35,252,155	0.16	*PARD3*	Imatinib	Unknown
10	11q24	122000841	122217796	0.22	*STS-1 / CBL* interacting protein	Imatinib, dasatinib, HU	CML in hematologic remission
4-R	15q21	48682473	48832856	0.15	*TRPM7, SPPL2A*	Imatinib	CML in hematologic remission
42-R*	17q	33318471	47862514	14.54	*STAT3, STAT5A/B*	HU, imatinib	CML in acceleration
45-R*	17q	37042201	78599918	41.55	*STAT3, STAT5A/B*	Imatinib	Unknown
46-R*	17p-q	18857962	78599918	59.74	*STAT3, STAT5A/B*	Unknown	CML
21-R	20p13	1658272	1861481	0.2	*SIRPA*	Imatinib	Unknown
27	22q11	14441016	21947838	7.51	Duplication *BCR*	Imatinib	Unknown
16	22q11	14441016	21947838	7.51	Duplication *BCR*	HU, imatinib	CML
24	22q11	14441016	21947838	7.51	Duplication *BCR*	Imatinib	Unknown
5	22q11	14441016	21947838	7.51	Duplication *BCR*	Imatinib	CML
33-R*	22q11	14441016	21947838	7.51	Duplication *BCR*	DA, imatinib	Myeloid blast crisis
40-R*	22q11	14441016	21960478	7.51	Duplication *BCR*	Imatinib	Myeloid blast crisis
34	22q11	14441016	21947838	7.51	Duplication *BCR*	HU, ICT, imatinib, dasatinib	Lymphoid blast crisis
33-R*	X	1	154824264	154	Trisomy X	DA, imatinib	Myeloid blast crisis

Copy number alterations (CNAs) in TKI-resistant samples without a matched diagnostic sample are shown with plain numbers in the column "ID." CNAs in TKI-resistant samples with a matched diagnostic sample with the CNAs being present in both the diagnostic sample and the resistant sample are shown with numbers + "R" in the column "ID." CNAs which were newly acquired in matched resistant samples compared to the diagnostic samples are shown with numbers + "R*" in the column "ID."

ID indicates patient ID; Starting position, start position of genomic lesion; Ending position, end position of genomic lesion; HU, hydroxyurea; DA, daunorubicin plus cytarabine; ICT, induction chemotherapy; and IFN, interferon-α.

in the UCSC Genome Browser (http://genome.ucsc.edu/) databases, a total of 36 deletions (Table 1), 29 duplications (Table 2), and 9 regions of copy number neutral LOH (Table 3) were identified by SNP array analysis.

Recurrent lesions were detected on chromosomes 1, 8, 9, 17, 19, and 22. On chromosome 1p36, 1 resistant sample displayed a submicroscopic deletion (sample 2) and 2 serial samples from other persons showed a common region of UPD (samples 41, 41-R and 43 and 43-R; Tables 1,3; supplemental Figure 3). These lesions of UPD were present in both the diagnostic sample and the TKI-resistant sample. Therefore, they are probably not associated with TKI resistance but confirm previous reports of frequent LOH in this region in CML,[8] which may play a role in the initial development of CML. In search for possible mutations, we sequenced the candidate genes arginine-glutamic acid dipeptide (RE) repeats (*RERE*) and enolase 1 (*ENO-1*), which were contained in the microdeletion in sample 2. However, apart from a 12-bp insertion in exon 20 of the *RERE* gene (supplemental Figure 3), which probably can be interpreted as a rare polymorphism or genomic

Table 3. Copy number neutral LOH detected in resistant CML samples

ID	Chromosome	Starting position	Ending position	Length, Mb	Candidate genes	Treatment	Disease status
41-R	1p36	825,852	18193698	17.36	*RERE, ENO-1*	Imatinib	Unknown
43-R	1p36	18,323,554	52881039	34.55	*RERE, ENO-1*	Imatinib	Unknown
5	2q	182983286	242712341	59.73		Imatinib	CML
31	3q	123593611	136239458	12.65		HU, imatinib	CML in hematologic remission
36-R	5p	29256280	35121357	5.87		HU, imatinib	CML in hematologic remission
41-R	6q	109232515	124242464	15.01	*FYN*	Imatinib	Unknown
36-R	6q	139970070	153644361	13.67		HU, imatinib	CML in hematologic remission
41-R	11p15	8895510	19285890	10.39		Imatinib	Unknown
42-R*	17q	23581904	78599918	55.01	*STAT3, STAT5A/B*	HU, imatinib	CML in acceleration
27	19q13	58448005	63731511	5.28	*BIRC8, AURKC*	Imatinib	Unknown
10	19q13	38496239	63700378	25.2	*BIRC8, AURKC*	Imatinib, dasatinib, HU	CML in hematologic remission

Copy number alterations (CNAs) in TKI-resistant samples without a matched diagnostic sample are shown with plain numbers in the column "ID." CNAs in TKI-resistant samples with a matched diagnostic sample with the CNAs being present in both the diagnostic sample and the resistant sample are shown with numbers + "R" in the column "ID." CNAs which were newly acquired in matched resistant samples compared to the diagnostic samples are shown with numbers + "R*" in the column "ID."

ID indicates patient ID; Starting position, start position of genomic lesion; Ending position, end position of genomic lesion; HU, hydroxyurea; DA, daunorubicin plus cytarabine; ICT, induction chemotherapy; and IFN, interferon-α.

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abnormality, we found no gene disrupting mutations in the remaining alleles of these genes.

Chromosome 17 was most heavily affected by secondary genomic alterations on development of TKI resistance. Four of the 20 serial samples showed newly acquired genomic alterations on chromosome 17. Changes composed either large deletions of chromosome 17p or large duplications or UPD of chromosome 17q (Tables 1-3; supplemental Figure 4). Genomic disruptions occurring on chromosome 17 are one of the most common known changes arising during disease progression.[9] In some cases, deletions of chromosome 17p have been found to contain inactivating mutations of *p53*.[10] Of note, in samples 42-R and 45-R, the breakpoints leading to duplication of chromosome 17q lie in close proximity to the *STAT3* and *STAT5A/B* genes.

On chromosome 19q, 2 patients displayed a common region of acquired UPD (19q13.32-19q13.43; Table 3).

On chromosomes 9 and 22, deletions flanking the *ABL1* and *BCR* genes were found in 5 patients. These are deletions of the reciprocal *ABL1-BCR* fusion product, which are known to occur in up to 10% to 17% of CML cases and have an effect on prognosis of patients treated with either hydroxyurea or interferon-α[11] but not with imatinib.[12] These deletions have been characterized with conventional cytogenetic methods[13] showing that the size of the deletions affects prognosis. However, a tumor suppressor gene has not been identified. In 3 of our 5 patients, the deletions on chromosome 9 spanned a common 1.9-Mb region centromeric to *ABL1*. One of the genes in the vicinity to the common breakpoint in this deleted region was protein phosphatase 2A activator, regulatory subunit 4 (*PPP2R4*), an activator of protein phosphatase 2A (*PP2A*), which was recently shown to be suppressed in imatinib-resistant CML.[14] *PPP2R4* therefore appeared as a candidate tumor suppressor gene, and we sequenced all exons of this gene in the patients with 9q deletions. However, no alteration from the reference sequence was detected in the remaining allele. On chromosome 22q11, the boundaries of the reciprocal deletions were heterogeneous; and in 2 cases, they began clearly telomeric to the *BCR* gene (Table 1). These SNP array results can be explained by duplication and insertion of the *BCR-ABL1* fusion gene in a situation of a deleted reciprocal *ABL1-BCR* fusion product as evidenced by cytogenetic analysis (supplemental Table 1). Because the deletions of the reciprocal *ABL1-BCR* fusions are already detectable in the diagnostic samples, they are probably not associated with secondary resistance against TKIs.

Another accumulation of common lesions detectable on chromosome 22q11 were heterozygous deletions in the immunoglobulin lambda constant 1 (*IGLC1*) locus. These deletions were detectable in 2 resistant samples without a paired diagnostic sample and in 1 serial resistant sample on development of TKI resistance (Table 1; supplemental Figure 5). Therefore, the acquisition of these deletions is probably associated with TKI resistance or disease progression. Deletions of the IGLC1 locus are occasionally observed in B-cell acute lymphoblastic leukemia,[15] resulting from λ light chain rearrangements. Prior investigators have shown that lymphoid blast crisis of CML displays a similar genomic profile to de novo Philadelphia chromosome-positive acute lymphoblastic leukemia.[16] Furthermore, recent data suggested that transition from chronic phase CML to lymphoid blast crisis and drug resistance involved activation of the lymphoid transcriptional programs, such as up-regulation of the lymphoid transcription factor *PAX5* and the activation-induced cytidine deaminase (*AID*).[17] This uncovered a causative role of AID in the acquisition of *BCR-ABL1* mutations and increased genomic instability in the progression of CML. Most importantly, imatinib treatment of *PAX5*-transduced CML cells led to the selection and outgrowth of $CD19^+$ CML subclones, which showed evidence of de novo immunoglobulin rearrangement indicating RAG1/RAG2 activity. Therefore, finding *IGLC1* deletions on development of TKI resistance in our CML samples corroborates these findings and fits well with the clinical observation of lymphoid blast crisis, and interestingly, also myeloid blast crisis with concomitant TKI resistance in these patients.

In conclusion, our high-density SNP array analysis identified new submicroscopic genomic lesions in 26 of 45 TKI-resistant CML patients. The resulting mean of 1.68 copy number alterations per TKI-resistant patient is slightly higher than in SNP array data from chronic phase CML samples demonstrated by Mullighan et al[16,18] and less than the frequency of genomic lesions detected by higher-density SNP arrays carried out by Khorashad et al.[19] We did not observe a new unequivocal recurrent genomic lesion associated with TKI resistance. Nevertheless, in individual cases, our data identified interesting candidate genes in the context of TKI resistance. Moreover, the observation of acquired *IGLC1* deletions on TKI resistance corroborates recent findings[17] of a causative role of B-lymphoid transcriptional programs in the disease progression and acquisition of resistance against TKI therapy.

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Authorship

Contribution: D.N. designed research, performed SNP array analysis, and wrote the paper; S.O., M.K., and N.K. performed SNP array analysis; M. Müschen, R.P., N.H.T., and W.-K.H. analyzed data and edited the paper; A.M., V.N., H.S.K., M.-S.C., S.K., and M. Mossner performed mutation analyses; A.K., T.W., T.H., and C.H. designed research and acquired patient samples; and H.P.K. designed research, performed data analysis, and edited the paper.

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Errata

Subramanian KK, Jia Y, Zhu D, et al. Tumor suppressor PTEN is a physiologic suppressor of chemoattractant-mediated neutrophil functions. *Blood*. 2007;109(9):4028-4037.

In the supplemental data of the May 1, 2007, issue, the authors failed to cite the source material in the legend for Figure S1. The attribution with Figure S1 should have read: "The preceding is a reprint of Fig. 5A in Zhu, D. et al Deactivation of phosphatidylinositol 3,4,5-trisphosphate/Akt signaling mediates neutrophil spontaneous death. *Proc Natl Acad Sci U S A.* 2006;103(40):14836-14841. © 2006 National Academy of Sciences, U.S.A."

The error was corrected in the online "Supplemental Figures and Videos" data supplement.

Eberhard Y, McDermott SP, Wang X, et al. Chelation of intracellular iron with the antifungal agent ciclopirox olamine induces cell death in leukemia and myeloma cells. *Blood*. 2009;114(14):3064-3073.

On pages 3064 and 3072 of the October 1, 2009, issue, the 12th author's name is incorrectly listed without the middle initial and initialized as J.D. in the "Authorship Contribution." The correct name is John E. Dick and correct initials are J.E.D. The errors were corrected in the online version, which now differs from the print version.

Salles II, Thijs T, Brunaud C, et al. Human platelets produced in nonobese diabetic/severe combined immunodeficient (NOD/SCID) mice upon transplantation of human cord blood CD34$^+$ cells are functionally active in an ex vivo flow model of thrombosis. *Blood*. 2009; 114(24):5044-5051.

On page 5044 in the December 3, 2009, issue, in the second paragraph of the Introduction, there is an error in the second sentence that read, "Such produced platelets are functional, as demonstrated in aggregation assays and by expression of P-selectin on the platelet surface or by activation of glycosylphosphatidylinositol (GPI) IbIIIa upon thrombin/thrombin receptor-activating peptide (TRAP) stimulation.[6,11-14]" The text "glycosylphosphatidylinositol (GPI) IbIIIa" is incorrect. The sentence should have read: "Such produced platelets are functional, as demonstrated in aggregation assays and by expression of P-selectin on the platelet surface or by activation of GPIIb/IIIa upon thrombin/thrombin receptor-activating peptide (TRAP) stimulation.[6,11-14]"

Stewart KA, Richardson PG, San-Miguel JF. How I treat multiple myeloma in younger patients. *Blood*. 2009;114(27):5436-5443.

Several authors' proof corrections were not included in the original publication of the December 24, 2009, "How I treat" article cited above. The editors and staff of *Blood* apologize to the authors and readers for this error.

On page 5436, in the second sentence of the "Abstract," the parenthetical phrase "(and a potential "operational cure" in some)" should have been deleted. The sentence should have read: "A reasonable goal of MM treatment in younger "transplant eligible" patients is to initiate therapy with a target goal of durable complete remission, and the anticipated consequence of long-term disease control."

On pages 5440 and 5441, citations 54 through 70 did not correspond with the correct sources on the References list, and the last 2 references (ie, 71 and 72) were not cited in text at all.

Additional minor edits to text were made by the authors. The errors have been corrected and the entire article is republished correctly in the online version, which now differs from the print version as originally published.

Readers are advised to download the corrected and republished online version that replaces the previously posted version.

Nowak D, Ogawa S, Müschen M, et al. SNP array analysis of tyrosine kinase inhibitor-resistant chronic myeloid leukemia identifies heterogeneous secondary genomic alterations. *Blood*. 2010;115(5):1049-1053.

On page 1049 of the February 4, 2010, issue, the 12th author's last name is spelled incorrectly as Thoenissen. The correct spelling is Thoennissen. The errors were corrected in the online version, which now differs from the print version.