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Genetic Mutations in a Patient with Chronic Myeloid Leukemia Showing Blast Crisis 10 Years After Presentation

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Abstract. *Since the introduction of tyrosine kinase inhibitors (TKI), the prospects for patients with chronic myeloid leukemia (CML) have improved significantly. Herein we present the case of a patient with CML who experienced blast crisis and development of acute myeloid leukemia (AML) 10 years after presentation. The CML was characterized by the gene fusion of breakpoint cluster region BCR and tyrosine-protein kinase ABL1. During treatment different therapeutic protocols including imatinib, nilotinib, dasatinib and ponatinib were applied due to development of resistance or non-response. Fluorescence in situ hybridization (FISH) and next-generation sequencing (NGS) were used to describe cytogenetic and molecular aberrations elucidating the development into AML: A loss of chromosome 7, as well as an arising frequency of variants in the gene met proto-oncogene MET (p.T110I) and tyrosine-protein phosphatase non-receptor type 11 PTPN11 (p.Q510L) was observed. This report describes the comprehensive characterization of a clinical case showing multiple therapeutic resistances correlated with genetic aberrations.*

Most patients with chronic myeloid leukemia (CML) show very good response towards therapy protocols with tyrosine kinase inhibitors (TKI) such as imatinib or nilotinib (1). However, some patients develop resistance to the standard regimen during their clinical course (2). This requires a subsequent change in treatment due to the risk of a blast crisis (3). Furthermore, during the past decade the identification of mutations by next-generation sequencing (NGS) has become a key element in cancer research, allowing sophisticated molecular diagnostics, as well as comprehensive retrospective molecular analysis of interesting cases. Thus, NGS provides the possibility to retrospectively elucidate molecular mechanisms underlying different drug responses, as well as drug resistance mechanisms, clonal development and evolution. Linking NGS data with conventional diagnostic tools such as cytogenetics, fluorescence *in situ* hybridization (FISH) and their correlation with therapeutic intervention and observed response allows deeper understanding of individual cases.

Here the comprehensive characterization of a clinical case showing multiple types of therapeutic resistance correlated with genetic aberrations is described.

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Case Report

A man (born in 1965) who was diagnosed with CML in May 2004 presented with a blast crisis and acute myeloid leukemia (AML) in February 2015. Conventional diagnostic analyses were performed by cytogenetics and FISH. From March 2015, additional NGS was routinely and retrospectively performed monitoring the initial CML as well as the AML manifestation. Thereby, a comprehensive analysis and evaluation of therapeutic interventions as well as the diagnostic measurements were performed. Over the 10 years analyzed, different therapeutic protocols were used. Therapeutic intervention, NGS analysis and cytogenetic diagnostics are summarized in Figure 1.

NGS. DNA for NGS analyses was isolated from nucleus suspensions of bone marrow (Nucleospin Tissue XS; Macherey Nagel, Düren Germany), peripheral blood smears (Nucleospin Tissue, Macherey Nagel) and peripheral blood (NucleoBond CB 100, Macherey Nagel) performed according to the manufacturer's instructions. NGS analysis was performed using Ion AmpliSeq™ Cancer Hotspot Panel

v2 (Thermo Fisher Scientific, Schwerte, Germany) and Ion PGM™ System (Thermo Fisher Scientific). This panel includes 50 genes associated with different cancer types. Furthermore, NGS data were mapped against the human genome (hg19) and analyzed by Ion Reporter™ Software (Thermo Fisher Scientific, Carlsbad, CA, USA). Only exonic variants with a quality over 60 (Phred-Score) and amplicon coverage above 500 are shown in Figure 1. ClinVar (National Center for Biotechnology Information, National Library of Medicine, Rockville Pike, Bethesda MD, USA) and Functional Analysis Through Hidden Markov Models (FATHMM) prediction were used for data interpretation (4).

Cytogenetics and molecular cytogenetics. Chromosomal analysis on bone marrow was performed using RC-banding (samples until 2009) (5) and GTG-banding (samples after 2009, Department of Human Genetics, University Bremen). A minimum of 20 metaphase cells were analyzed and the karyotype was described according to the 2016 International System for Human Cytogenetic Nomenclature (6).

FISH using the following probes was carried out according to the manufacturers' instructions and 100 interphases (sample August 2014: 300) were routinely counted: Vysis D20S108 (20q12) FISH Probe (Abbott Molecular, Abbott Park, IL, USA); Vysis D7S486/Vysis CEP 7 FISH Probes (Abbott Molecular), Vysis LSI BCR/ABL Dual Color, Dual Fusion Translocation Probe and XCE 8 Blue (MetaSystems, Altlussheim, Germany).

Breakpoint cluster region (BCR) and tyrosine-protein kinase (ABL1) detection. *BCR-ABL* status was routinely determined according to standard methods (polymerase chain reaction) at the Department of Internal Medicine, Medical Clinic C, Clinic for Hematology, Oncology and Palliative Care, University Medical Center Greifswald. Values are presented in international standard. Bone marrow and peripheral blood were first characterized by cytogenetics and FISH in May 2004.

Clinical course. In May 2004, the patient was found to be *BCR-ABL*-positive [*BCR-ABL* 117% peripheral blood, karyotype 46XY,t(9;22)(q34;q11)] and was treated with hydroxycarbamide according to the standard protocol. Hydroxycarbamide was replaced with imatinib (200 mg per day) in July 2004. In September 2004, the imatinib dose was increased to 400 mg daily due to persisting 14% *BCR-ABL* (peripheral blood). Peripheral blood screening revealed 45% *BCR-ABL* in November 2005 and the imatinib dose was further increased to 600 mg daily. Despite dose adaption for a further 2 months (January 2006), a further increase of *BCR-ABL* to 58% in bone marrow and 28% in peripheral blood required a subsequent dose escalation of imatinib to 800 mg per day. Corresponding cytogenetic analyses revealed an additional aberration with a subpopulation of 48,XY,+8,t(9;22)(q34;q11),+der(22)t(9;22).

In March 2006, the patient was enrolled in a study (CAMN107A2101; registered at http://www.clinicaltrials.gov as NCT00109707) (7) and received nilotinib at 800 mg per day. The patient showed a major molecular response of *BCR-ABL* for 8 years (March 2014) accompanied by several side-effects such as gastrointestinal discomfort and permanent headache.

Loss of major molecular response (*BCR-ABL* 27% in peripheral blood) in March 2014 led to a change of treatment protocol replacing nilotinib by dasatinib (100 mg per day). Due to side-effects such as nausea and vomiting, the treatment was stopped. In August 2014 bone marrow analysis revealed 10% blasts and 31% *BCR-ABL*. Therefore, therapeutic intervention was changed to ponatinib (45 mg daily).

Ponatinib treatment (6 months) failed to induce a major molecular response (*BCR-ABL* 19% bone marrow) and a transition into blast crisis occurred in February 2015 (70% blasts in peripheral blood). Additional FISH analysis revealed a deletion of chromosome 7 [+del(7)]. A decrease of mapped reads for chromosome 7 was also revealed by NGS from 11.3% initially, to 9.1% in August 2014 and 6.8% in February 2015.

Therefore, a cytoreductive therapy with cytarabine for 5 days was initiated, followed by one cycle of mitoxantrone, cytarabine and fludarabine (MitoFlaG) due to lack of response. Despite this intense therapy, the blasts were still persistent in the peripheral blood (until March 2014). Therefore a novel induction scheme (azacitidine with bosutinib) (8) was introduced resulting in rapid hematological complete remission (CR).

NGS analyses were performed retrospectively on four samples as well as prospectively on samples of different origins. During the whole time (2004-2015) homozygous (frequency 100%) variants in platelet-derived growth factor receptor alpha (*PDGFRA*), harvey rat sarcoma viral oncogene homolog (*HRAS*), rearranged during transfection (*RET*), fibroblast growth factor receptor 3 (*FGFR3*) genes and heterozygous (frequency 50%) variants in *ABL1* and *RET* were revealed. Except for *ABL1* (p.K247R), none of the variants lead to an amino acid change (Table I). However, the *RET* gene (c.2307G>T) and ABL1 (p.K247R) variants are classified as pathogenic according to FATHMM. Despite the fact that *RET* (c.2307G>T) does not lead to an amino acid change, this mutation was frequently found in hematopoietic neoplasms (COSM4418405) (9).

A single nucleotide variant (SNV) affecting the proto-oncogene *MET* (p.T1010I) was detected with a frequency of nearly 25% in the initial samples of 2004. During disease progression, this frequency increased to more than 90% in March 2014. *MET* encodes a proto-oncogene and receptor tyrosine kinase located on chromosome 7. The SNV leads to a nucleotide and amino acid change (p.T1010I), representing a known mutation (COSM707) predicted as pathogenic (FATHMM score 0.98). ClinVar also described this mutation

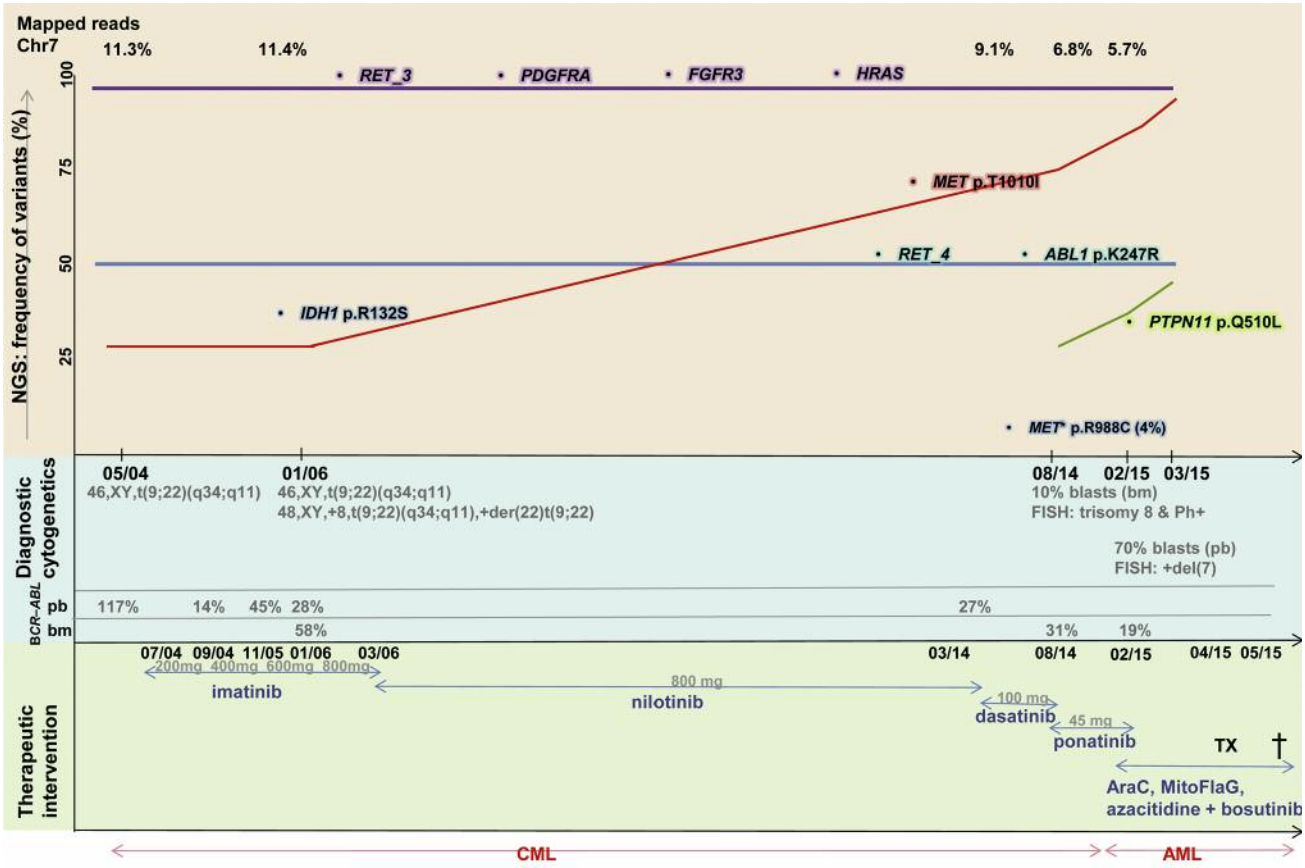

Figure 1. *Next-generation sequencing, diagnostic cytogenetics and therapeutic intervention. pb: Peripheral blood; bm: bone marrow; TX: transplantation; Chr: chromosome, AML: acute myeloid leukemia, CML: chronic myeloid leukemia, NGS: next-generation sequencing, FISH: fluorescence in situ hybridization, t: translocation, der: derivative chromosome, AraC: cytarabine, MitoFlaG: mitoxantrone, cytarabine and fludarabine, PDGFRA: platelet derived growth factor receptor alpha, HRAS: harvey rat sarcoma viral oncogene homolog, RET: rearranged during transfection, FGFR3: fibroblast growth factor receptor 3, BCR: breakpoint cluster region, ABL1: tyrosine-protein kinase, MET: proto-oncogene met, PTPN11: tyrosine-protein phosphatase non-receptor type 11, IDH1: isocitrate dehydrogenase 1.*

Table I. *Detected gene variants and their allelic frequency, location, single nucleotide variant (SNV) description, coding, amino acid change and predictive score (FATHMM).*

Occurrence	Gene	Allelic frequency	Location	SNV	Coding	Amino acid change	Allele name	FATHMM (score)
Persisting	*PDGFRA*	100%	NM_006206.5	rs1873778	c.1701A>G	–	Novel	N.A.
	HRAS	100%	NM_001130442.1	rs12628	c.81T>C	–	COSM249860	Neutral (0.07)
	RET	100%	NM_020975.4	rs1800861	c.2307G>T	–	COSM4418405	Pathogenic (0.79)
	FGFR3	100%	NM_001163213.1	rs7688609	c.1959G>A	–	Novel	N.A.
	RET	50%	NM_020975.4	rs1800863	c.2712C>G	–	COSM3751779	Neutral (0.27)
	ABL1	50%	NM_007313.2	rs34549764	c.740A>G	p.K247R	COSM6005485	Pathogenic (0.92)
Increasing	*MET*	25-90%	NM_001127500.1	rs56391007	c.3029C>T	p.T1010I	COSM707	Pathogenic (0.98)
	PTPN11	30-50%	NM_002834.3	rs121918470	c.1529A>T	p.Q510L	COSM1318059	Pathogenic (0.99)
Punctual	*MET*	4%	NM_001127500.1	rs34589476	c.2962C>T	p.R988C	NOCOSMIC988	N.A.
	IDH1	43%	NM_005896.3	rs121913499	c.394C>A	p.R132S	COSM28748	Pathogenic (0.92)

– : No amino acid change, N.A.: not available, SNV: single nucleotide variant, FATHMM: Functional Analysis Through Hidden Markov Models, *PDGFRA*: platelet derived growth factor receptor alpha, *HRAS*: harvey rat sarcoma viral oncogene homolog, *RET*: rearranged during transfection, *FGFR3*: fibroblast growth factor receptor 3, *ABL1*: tyrosine-protein kinase, *MET*: proto-oncogene met, *PTPN11*: tyrosine-protein phosphatase non-receptor type 11, *IDH1*: isocitrate dehydrogenase 1.

as likely pathogenic in somatic cells. Furthermore, it was found in tumor samples of lung and thyroid (10, 11).

The isocitrate dehydrogenase 1 (*IDH1*) gene was found to be affected (frequency 43%) by a known SNV (COSM28748) predicted as pathogenic (FATHMM score 0.92) exclusively in a sample from 2006. *IDH1* mutations are common in AML (12) and this mutation was also found in patients with AML and multiple myeloma (13, 14). A variant in protein tyrosine phosphatase, non-receptor type 11 (*PTPN11*, frequency 30%) and a second variant in *MET* (4% frequency) were detected in the sample of August 2014. The frequency of the *PTPN11* SNV had increased to 50% by March 2015. This variant results in a nucleotide exchange (c.1529A>T) and amino acid change (p.Q510L). This missense mutation is known (COSM1318059), classified as pathogenic (FATHMM prediction score 0.99) and was found in AML samples (15). The variant of the *MET* gene was at position 116411923 and leads to a nucleotide (c.2962C>T) and amino acid change (p.R988C). ClinVar describes this mutation as likely pathogenic in somatic cells (16).

In March 2015, the patient immediately received full matched allogeneic stem cell transplantation with myeloablative conditioning (busulfane, cyclophosphamide). Unfortunately, the patient developed severe infection complications on day 21 and died on day 28.

Discussion

In this article, we present the case of a patient with CML who did not respond to different therapeutic interventions and eventually developed a blast crisis and AML 10 years after diagnosis. Therapeutic protocols had to be changed due to non-response or secondary drug resistance and development of *BCR-ABL*-positive cells. NGS analysis revealed silent mutations in the *PDGFRA, HRAS, RET* and *FGFR3* genes and a point-mutation in *ABL1* from the initial diagnosis until blast crisis in 2015. *ABL1* (p.K247R) was found in samples from patients with CML who failed to achieve response to imatinib (17), and on the other hand, it is described as a polymorphism (17, 18). Additionally, we detected an SNV of the *MET* gene with an initial frequency of nearly 25%, rising to 90% in March 2015. This transmembrane tyrosine kinase promotes *e.g.* cell proliferation and survival *via* phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathway signaling (19). The detected SNV p.T1010I is located on the intracellular juxtamembrane domain and may contribute to persistent pathway activation and tumor pathophysiology (20). This SNV was found in breast (20) and lung cancer (21), Ewing's sarcoma (22) and other cancer types (23). Thus, therapeutic intervention with a MET inhibitor may have had beneficial effects for the patient. However, this mutation is also described as a potential rare polymorphism (23, 24).

Despite increasing the dose of imatinib, the patient never achieved CR. During that time, the patient developed more cytogenetic anomalies and an additional clone with +8 and a second Philadelphia chromosome. These chromosomal anomalies are most frequent in CML (25-27). It seems that increased expression of *BCR-ABL* does play a critical role in promoting genetic instability (3). *BCR-ABL* regulates DNA damage and response by accumulation of further DNA lesions. As many as 10% of patients who respond to imatinib finally developed additional karyotype abnormalities, among others, trisomy 8 (28). Additionally, a variant in the *IDH1* (p.R132S) was detected. Most common variants are heterozygous mutations at p.R132 (29) and this variant was found in a patient with AML (30).

In general, 82% of patients achieve CR with imatinib, but some patients are non-responders (31). Hence therapy of our patient was changed to second-generation TKI nilotinib. This ABL inhibitor is used after failure of imatinib therapy and induces CR in approximately 50% of patients (31). The patient also showed complete cytogenetic response, but after 8 years of CR with nilotinib, the frequency of *BCR-ABL* rose again and the therapy was changed to dasatinib. Dasatinib is an ABL inhibitor which targets more kinases in comparison to imatinib and nilotinib (2). The patient responded to dasatinib, but therapy had to be terminated because of several side-effects. In August 2014, FISH diagnosis revealed the same results as in 2006. It has been demonstrated that treatment with nilotinib or dasatinib leads to improved response rates and reduces the rate of transformation in patients in comparison to imatinib (31). However, NGS analysis detected a SNV in *PTPN11*, a rise of the previously described *MET* mutation, but the *IDH1* variant was missing. *PTPN11* variant is a known driver mutation in AML (32-36). Therapeutic intervention was changed to the third-generation TKI ponatinib. But ultimately the patient developed a blast crisis in February 2015 and therapy was changed to AML protocols. FISH diagnosis revealed monosomy 7 and NGS analysis detected a decrease of mapped reads of chromosome 7, which was already obvious in the sample of August 2014. Monosomy 7 was reported to correlate with risk of acute leukemia (37).

Apparently, during 10 years of therapeutic intervention, the patient gained additional mutations and clonal selection including genetic shifting occurred. Monosomy 7, as well as *MET* (p.T1010L) and *PTPN11* (p.Q510L) variants, are especially interesting points. Detection of these findings at an earlier point of time could have increased the chance of recovery of the patient *e.g.* by use of a MET inhibitor or earlier stem cell transplantation. Thus, this case report shows that the early comprehensive integration of detailed molecular diagnostics can provide significant diagnostic value as well as being helpful for therapeutic protocol decisions.

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