Chemotherapy: Open Access
ISSN: 2167-7700

Cheng et al., Chemo Open Access 2016, 5:2
DOI: 10.4172/2167-7700.1000193

Research Article Open Access

A New Mutation Identified in an Imatinib and Nilotinib Resistant Chronic Myeloid Leukemia Patient

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Received date: November 23, 2015; **Accepted date:** February 24, 2016; **Published date:** February 27, 2016

Abstract

Chronic myeloid leukemia (CML) is a heterogeneous disease induced by *BCR-ABL* oncogene. Since the emergence of Tyrosine kinase inhibitors (TKIs), treatment of CML has been drastically changed. However, resistance and intolerance of TKIs have frequently been reported, especially in advanced-stage patients. The most critical reason for TKIs resistance is point mutations within the *ABL1* kinase domain that interfere the binding of *ABL1* with TKIs. The types and frequencies of mutations have been reported in different population. In this study, we identified a new mutation A2387G (N796S) within the *BCR* domain (exon 10) via next generation exon sequencing in an imatinib and nilotinib resistant CML patient.

Keywords: Chronic myeloid leukemia; Tyrosine kinase inhibitors; Resistance; Mutation

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm induced by the *BCR-ABL1* fusion gene with an incidence of 1-2 cases per 100,000 and accounts for 15% of newly diagnosed cases of leukemia in adults [1]. As we all know CML often starts with a chronic phase, which is characterized by granulocytosis and splenomegaly [2]. CML was a fatal disorder until the introduction of TKIs, which revolutionized CML therapy, changing CML into a manageable chronic disease [3]. But TKIs resistance sometimes takes place; one of the most common mechanisms involves point mutations in the kinase domain of *BCR-ABL1*, which impairs the activity of the available TKIs. The T315I is one of the most important mutations, as it displays resistance to all currently available TKIs except ponatinib [4]. In the present study, we found a new mutation, N796S, in the *BCR* (exon 10) domain via next generation exon sequencing in a CML patient with T315I and F359V mutations. The response to therapy was evaluated mainly based on the measurement of hematologic, cytogenetic and molecular responses.

Materials and Methods

Patient and treatment

The patient was treated with imatinib at a dose of 400 mg every day as frontline treatment started from diagnosis. After five-month-treatment, this patient advanced into acute B-lymphoid leukemia. Directly sequencing discovered two mutation points, T315I and F359V within the *ABL1* kinase domain in bone marrow samples. The patient was processed to allogeneic hematologic stem cell transplantation (allo-HSCT) after one cycle cytarabine-based induction and three cycles daunorubicin-based consolidation therapies, and ponatinib at a dose of 45mg/d was administered during treatment. The myeloablative conditioning regiment for allo-HSCT consisted of the following agents: Cytarabine (2.5 mg/m^2/day, intravenously) on day-10 and -9, busulfan (6.4 mg/kg, intravenously) on day-8 to -6; cyclophosphamide (3.6 g/m^2/day, intravenously) on day-5 and -4; antithymocyte globulin (250 mg/m^2/day, intravenously) on day-4 to -1 and semustine (500 mg/m^2, orally) on transplantion day. The patient received cyclosporine A, mycophenolate mofetil and short-term methotrexate for graft versus host disease prophylaxis [5]. The patient and the unrelated donor were fully HLA-matched. The total infusion count of cells was 5.98 × 10^8 mono-nuclear cells/kg or 4.08 × 10^6 CD34+ cells/kg according to recipient body weight. Unfortunately the relapse occurred in the patient after treated with allo-HSCT for two months, then chemotherapy and donor lymphocyte infusion were used for the patient. However, the relapse happened to the patient again.

RNA extraction and Real-time PCR

The levels of *BCR-ABL-210* and donor chimerism were detected by Real-time PCR based on Taqman probe. Total RNA was extracted from bone marrow by using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufactuerer's instructions. All reactions were conducted with reagents from Yuanqi Bio-Pharmaceutical (Shanghai, China) on the ABI 7500 real-time PCR machine (Applied Biosystems, Foster City, CA, USA).

Next generation exon sequencing and validation

Genomic exons mutation were detected by next generation exon sequencing and validated by DNA directly sequencing. Genomic DNA was isolated by using DNAzol (Invitrogen), and DNA purity was identified by electrophoresis. Ion AmpliSeq™ Exome Kit (Cat. no. 4487084, Life Technologies, Grand Island, NY, USA) was used for next generation sequencing analysis on the Ion Proton™ System. Coverage analysis was carried out firstly, and then *ABL1* and *BCR* exons analysis were performed, respectively.

The results of next generation exon sequencing were validated via DNA directly sequencing. Primers were as follows:

Chr9-F: 5'-AGTCTCAGGATGCAGGTGCT-3',

Chr9-R: 5'-TTGCCAGGAGCCTAGTGTTT-3', product size: 497bp,

Chr22-F: 5'-GCAAAACGCAGCAGTATGAC-3'

Chr22-R: 5'-AGCTTACAGGGGTCCCAGAG-3', product size: 300bp.

The analysis of sequencing applied with ABI Variant Reporter (V1.1) and Technelysium Chromas (V2.3) software.

Results

Acute B-lymphoid leukemia took place in the patient treated with imatinib for 5 months. Directly sequencing revealed F359V and T315I mutations in *BCR-ABL1* kinase domain, which was associated with TKIs resistance. Chemotherapy was performed until bone marrow morphology complete remission. Then the patient was processed to allo-HSCT and simultaneously administered with ponatinib.

Up to now, most laboratories use an unbiased direct DNA sequencing approach to screen for the presence of kinase domain mutations [6], thus the next generation exon sequencing was used for this patient's genomic DNA. The level of *BCR-ABL*^{IS} and donor chimerism were monitored after allo-HSCT until the patient death (Figure 1). DNA was extracted from patient's leukemic cells which were isolated from the bone marrow sample and identified by electrophoresis (Figure 2) for next generation exon sequencing and validation. Coverage analysis was carried out (Table 1), and we found T315I (C944T) and F359V (T1075G) point mutation within *ABL1* kinase domain (exon 6), and an unreported point mutation, N796S (A2387G) within the *BCR* domain (exon 10) via software analysis. Then this newly found mutation was validated by DNA directly sequencing (Figure 3). This patient ultimately experienced relapse again with hematopoiesis failure and pulmonary infection, and eventually died of disease 24 months later.

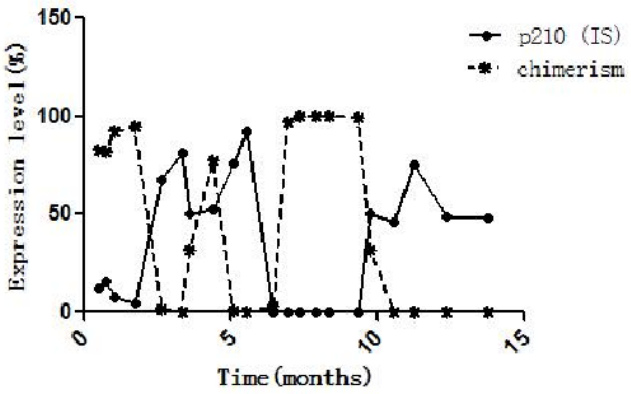

Figure 1: The results of fusion gene (p210IS) and donor chimerism every time after allogeneic stem cell translation. IS: International standardization.

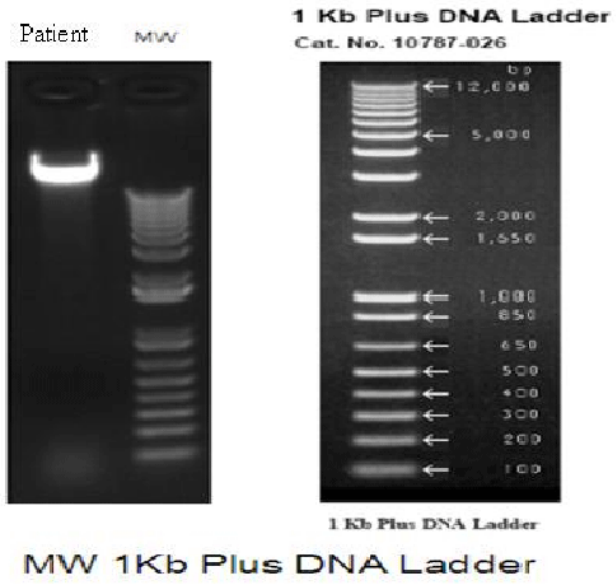

Figure 2: Identification of DNA quality by electrophoresis.

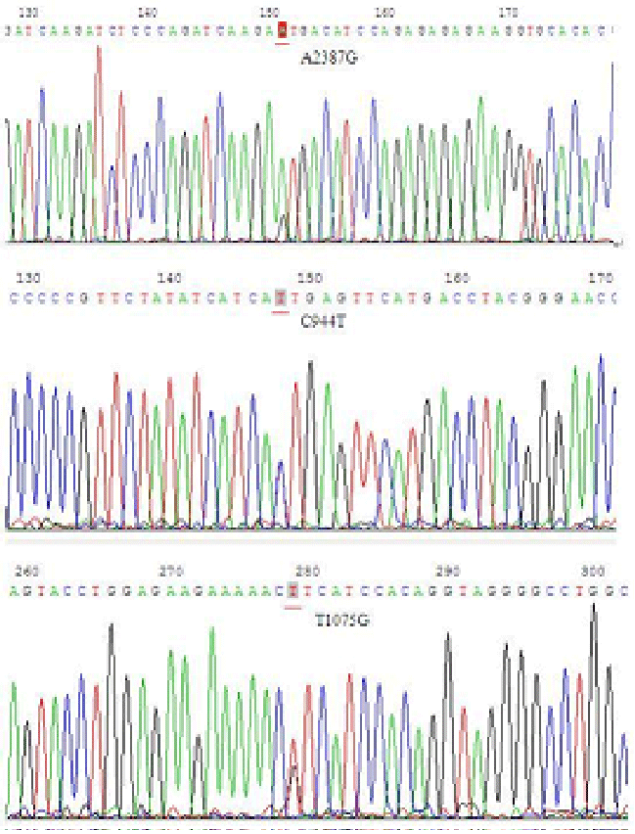

Figure 3: Validation of A2387G (N796S), C944T (T315I) and T1075G (F359V) point mutations via DNA directly sequencing.

Discussions

Many TKIs-resistance mechanisms has been discovered which can include *BCR-ABL*-dependent and independent mechanisms. Understanding the underlying reasons for resistance is an extremely significant step towards CML patients with TKIs resistance [7]. The resistance to TKIs can result from genomic instability in CML leukemia cells which is responsible for accumulation of point mutations in *BCR-ABL1* kinase domain. Point mutations mainly originate from enhanced oxidative DNA damage [8] or deregulated mechanisms of DNA repair [9]. Oxidative deamination of cytosine generates many mutagenic U: G mismatches that, if not repaired, would change from G:C to A:T, which is the most important point mutations in human tumors [10]. Furthermore, mitochondria DNA damage and repair may change the sensitivity of CML cells to TKIs [11]therefore the new revealed mutation in this patient, N796S, possibly plays a critical role in CML resistance to TKIs.

Analyses of patients' resistance to TKIs have identified more than 100 distinct point mutations in *BCR-ABL* domain at present [12]. Kim et al. observed a high frequency of mutation (63%) among their imatinib resistant CML patients [13]. However, most of the mutations do not occur with a high level of frequency in CML patients, and 15 mutations comprise approximately 85% of all those patients detected with mutations [14,15]. Recently, Jiang et al. reported that Patients with Philadelphia-positive leukemia with Y253H or F359V mutation were independent predictors of developing new mutations [16]. A minority of CML patients in chronic phase and advanced phase are either initially refractory to TKIs or develop T315I resistance. Therapeutic strategies including TKIs dose escalation, participating in clinical trial and allogeneic stem cell transplantation in eligible patients are needed to take into account for the treatment or prevention of resistance and disease progression [17], because T315I mutation confers cross-resistance to TKIs (imatinib, nilotinib, and dasatinib) *in vitro* and *in vivo* [18-20]. 2014 NCCN guideline definitely reports different treatment options for different mutations (Table 2) [21].

Barcode ID	Sample Name	Mapped Reads	On Target	Mean Depth
IonXpress_004	CRL-GW-exome	85,759,176	95.23%	250.6

Table 1: The results of coverage analysis by next generation exon sequencing.

For the patient with T315I and F359V mutation, third generation TKI ponatinib and allo-HSCT were performed. However, the relapse remainly occurred in the patient, and meanwhile a new mutation, N796S, was found in the patient's leukemic cells. Eventually the patient merely survived for 24 months. In a 2013 study by Khorashad et al. 1,700 patient samples with *BCR-ABL1* kinase domain mutations were analyzed using directly DNA sequencing. In this set of samples, 11.4% showed two or more mutations in the kinase domain [22]. At present, allo-HSCT is the only curative therapy for accelerated and blastic phase CML, and the overall cure rates are in the range of 15-40% and 5-20%, respectively [23].

Mutation	Treatment option
T315I	Omacetaxine, HSCT, or Clinical trial
V299L	Consider nilotinib or omacetaxine
T315A	Consider nilotinib, imatinib, bosutinib, omacetaxine
F317L/V/I/C	Consider nilotinib, or bosutinib, or omacetaxine
Y253H. E255K/V. F359V/C/I	Consider dasatinib, or bosutinib, or omacetaxine
Any other mutation	Consider high dose imatinib, dasatinib, nilotinib, bosutinib, or omacetaxine

Table 2: 2014 NCCN guideline about treatment options of CML with different mutations.

Conclusions

Although CML has acquired mostly progression on therapy, including the use of TKIs and allo-HSCT. But disease advance and relapse remain to be the main reasons for death. In the study, the patient with T315I, F359V and N796S mutations has poorer prognosis, which belongs to relapse and refractory leukemia. Nowadays, resistant mechanisms of T315I and F359V have been studied clearly, but the function of this new mutation, N796S, is unknown and needs further to be explored in TKIs resistance.

Acknowledgements and Declaration of Interests

Authors' contributions

HC and CH contributed equally to this work. HC detected samples, collected, analyzed and interpreted data, and wrote the manuscript; CH critically reviewed the manuscript and importantly given her advice in English; HQ, WZ, LC, and JY performed diagnosis and treatment for the patient.

Funding Support: The work was supported with the grants from National Natural Science Foundation of China (81400133), and the Shanghai Science and Technology Committee (15ZR1412600 to L.C).

Competing Interests

The authors declare that no potential conflicts of interest relevant to this article were reported.

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