BLOOD RESEARCH
VOLUME 51 · NUMBER 1
March 2016
ORIGINAL ARTICLE

A scientific treatment approach for acute mast cell leukemia: using a strategy based on next-generation sequencing data

Jeonghwan Youk[1#], Youngil Koh[1,2#], Ji-Won Kim[3], Dae-Yoon Kim[2], Hyunkyung Park[1], Woo June Jung[2], Kwang-Sung Ahn[2], Hongseok Yun[4], Inho Park[4], Choong-Hyun Sun[4], Seungmook Lee[4], Sung-Soo Yoon[1,2,5]

[1]Department of Internal Medicine, Seoul National University Hospital, [2]Cancer Research Institute, Seoul National University College of Medicine, [3]Department of Internal Medicine, Seoul National University Bundang Hospital, Seongnam, [4]Bioinformatics Group, Platform Development Center, CSP R&D, Samsung SDS, [5]ClinicalResearch Institute, Seoul National University Hospital, Seoul, Korea

p-ISSN 2287-979X / e-ISSN 2288-0011
http://dx.doi.org/10.5045/br.2016.51.1.17
Blood Res 2016;51:17-22.

Received on January 29, 2016
Revised on February 19, 2016
Accepted on March 2, 2016

[#]These authors contributed equally to this work.

[*]This research was supported by a grant of Korea Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI14C0072) and grant 03-2014-0230 from the SNUH Research Fund.

Correspondence to
Sung-Soo Yoon, M.D., Ph.D.
Department of Internal Medicine, Seoul National University Hospital, 101 Daehag-ro, Jongno-gu, Seoul 03080, Korea
E-mail: ssysmc@snu.ac.kr

Background
Mast cell leukemia (MCL) is the most aggressive form of systemic mastocytosis disorders. Owing to its rarity, neither pathogenesis nor standard treatment is established for this orphan disease. Hence, we tried to treat a patient with MCL based on the exome and transcriptome sequencing results of the patient's own DNA and RNA.

Methods
First, tumor DNA and RNA were extracted from bone marrow at the time of diagnosis. Germline DNA was extracted from the patient's saliva 45 days after induction chemotherapy and used as a control. Then, we performed whole-exome sequencing (WES) using the DNA and whole transcriptome sequencing (WTS) using the RNA. Single nucleotide variants (SNVs) were called using MuTect and GATK. Samtools, FusionMap, and Gene Set Enrichment Analysis were utilized to analyze WTS results.

Results
WES and WTS results revealed mutation in *KIT* S476I. Fusion analysis was performed using WTS data, which suggested a possible RARα-B2M fusion. When RNA expression analysis was performed using WTS data, upregulation of PIK3/AKT pathway, downstream of KIT and mTOR, was observed. Based on our WES and WTS results, we first administered all-trans retinoic acid, then dasatinib, and finally, an mTOR inhibitor.

Conclusion
We present a case of orphan disease where we used a targeted approach using WES and WTS data of the patient. Even though our treatment was not successful, use of our approach warrants further validation.

Key Words Leukemia, Mast cell, C-kit, Individualized medicine

INTRODUCTION

Mast cell leukemia (MCL) is the most aggressive form of systemic mastocytosis (SM). Common symptoms of MCL include flushes, fever, malaise, diarrhea, and tachycardia. Diagnosis of MCL requires that (i) SM criteria are fulfilled, and (ii) bone marrow (BM) atypical mast cells (MCs) comprise ≥20% of the total white blood cells (WBCs). MCL is composed of a leukemic variant (>10% in the peripheral blood) and an aleukemic variant (<10% in the peripheral blood). This orphan disease accounts for less than 1% of all SMs [1].

Owing to its rarity, the pathogenesis and standard treatment for MCL are not well established. According to Georgin-Lavialle *et al.*, most patients with MCL (83%) show a normal karyotype in conventional cytogenetic exams. At the molecular level, mutations in the *KIT* gene have been well investigated in MCL patients, even before the next-generation sequencing (NGS) era. A few therapeutic options for MCL are available, but no efficient treatment has been reproducibly validated. Thus, well-organized clinical trials should be primarily considered [1]. Without proper clinical trials, patients with mutations in *KIT* other than D816V or those with wild type *KIT* could be treated with an ABL

kinase inhibitor, such as imatinib. Unfortunately, ABL kinase inhibitors are not effective in patients with the *KIT* D816V mutation. Midostaurin, a multi-target protein kinase inhibitor, can be administered to patients with MCL regardless of mutations in *KIT*. Allogeneic stem cell transplantation may be a potential curative option for MCL, but a retrospective study found that the response rate was low (three-year survival rate=17% [2 of 12]) [2]. Traditionally, polychemotherapy, such as an AML-type induction regimen, has been used for cytoreductive therapy. Alternatively, steroids and interferon-α can be considered as treatment options.

With the exception of the *KIT* gene, molecular study and targeted therapy have not been thoroughly evaluated. Therefore, we attempted to treat a refractory MCL patient based on whole exome sequencing (WES) and whole transcriptome sequencing (WTS) of the patient's own DNA and RNA.

MATERIALS AND METHODS

An 18-year-old Korean female visited the Seoul National University Hospital with recurrent pain in the abdomen and both legs that lasted for 1 month. X-rays of the legs and an abdominal computed tomography (CT) scan were performed to determine the cause of the pain and revealed hepatomegaly with ascites and left inguinal lymphadenopathy (largest diameter: 2.4 cm). Excisional inguinal node biopsy revealed dense infiltrates of atypical MCs with strong C-KIT expression; however, no MCs were detected in the peripheral blood (white blood cell count: 7,570/L; 74.9% neutrophils, 21.0% lymphocytes, 3.6% monocytes, 0.4% eosinophils, and 0.1% basophils). We found an increase in immature MCs (24.1%) with bi-lobed nuclei in a BM smear. Most of the MCs showed atypical morphology. No morphological evidence of an associated hematopoietic non-mast cell lineage disease was found. The serum tryptase level was 425.0 μg/L. Chromosomal analysis showed a normal karyotype (46, XX [20]). In addition, we did not observe the *KIT* D816V mutation. One major and two minor criteria of SM established by the WHO were fulfilled. With the presence of 20% MCs on the BM smear, the patient was diagnosed with the aleukemic variant of acute MCL [3]. Liver dysfuncion was identified as a C finding of the disorder.

We initiated treatment with cytarabine (100 mg/m^2) for 7 days and idarubicin (12 mg/m^2) for 3 days, but the follow-up BM smear revealed persistence of MCL (MCs: 5.5% of total nucleated cells). Because the patient was reluctant to undergo allogeneic stem cell transplantation, we performed WES and WTS to find druggable mutations or activated signaling pathways.

Next-generation sequencing

BM blasts were acquired at diagnosis, and epithelial cells from saliva were obtained after induction chemotherapy. We used genomic DNA purification kits (Norgen Biotek Corp, Thorold, ON, Canada) to isolate the DNA. Quality was monitored by the NGS QC Toolkit (National Institute of Plant Genome Research, New Delhi, India). DNA was then fragmented for massively parallel sequencing via the HiSeq 2000 system (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. We used the SureSelect Human All Exon Kit (Agilent Technologies Inc., Santa Clara, CA, USA) for DNA capture. FASTQ files were aligned to the UCSC human reference genome (build hg19) using the Burrows-Wheeler Aligner (bwa-0.7.5a) [4] to generate a sequence alignment/map file. Next, the Genome Analysis Toolkit (GATK; Broad Institute, Cambridge, MA, USA) was used for local alignment [5]. Single nucleotide variants (SNVs) and small insertions and deletions (indels) were identified using GATK. SNVs were also identified using MuTect software (Broad Institute) [6]. CONTRA was used to determine copy number variations [7].

For RNA preparation, total RNA quality was assessed using the NanoDrop1000 spectrometer (Thermo Scientific, Wilmington, DE, USA). We used the TruSeq RNA library preparation kit for RNA isolation (Illumina, San Diego, USA). In brief, messenger RNA (mRNA) was purified using polyA selection, then chemically fragmented and converted into single-stranded cDNA using random hexamer priming. Next, the complimentary strand was generated to create double-stranded cDNA (ds-cDNA) that could be used for TruSeq library construction. The short ds-cDNA fragments were then connected with sequencing adapters, and suitable fragments were separated by agarose gel electrophoresis. Finally, TruSeq RNA libraries were built by PCR amplification, quantified using qPCR according to the qPCR Quantification Protocol Guide, qualified using the Agilent Technologies 2100 Bioanalyzer (Agilent Technologies, Palo Alto CA, USA), and then sequenced using the HiSe 2000 platform (Illumina, San Diego, USA). The sequencing data were aligned to genecode v18 by Tophat v1.0.12 (Tophat, genecode reference). Raw counts of mRNA were quantified using Samtools [8], and corresponding reads per kilobase per million reads (RPKM) were calculated using an in-house R script. We used RPKM value to determine differentially expressed genes. Fusion genes were analyzed by FusionMap [9]. To find upregulated intracellular signaling pathways, we used the Gene Set Enrichment Analysis (http://www.broadinstitute.org/gsea/msigdb/annotate.jsp, Broad Institute, MA).

RESULTS

WES analysis yielded a total of 226,007,918 mapped reads (BM DNA: 103,545,760; salivary epithelial DNA: 122,462,158). The mean read depth of the neoplasm was more than 100-fold. WES analysis using GATK failed to demonstrate noticeable nonsynonymous SNVs or small indels, whereas MuTect detected five SNVs, including *KIT* S476I (Table 1). Druggable genes associated with copy number changes were also not found. WTS analysis also found the *KIT* S476I mutation (Table 2). The possibility of RARα-B2M and RARα-ACTB fusion have been noted. When performing RNA ex-

Table 1. Single-nucleotide variations detected by whole-exome sequencing.

Gene	Position	Reference	Reference depth	Alternative	Somatic depth	Amino acid change
POTEE	2:132021766	A	42	C	4	K913T
KIT	4:55592103	G	131	T	6	S476I
MSH3	5:79950736	C	20	G	3	P64A
RRP7A	22:42910199	G	17	A	3	R224W
FAM3A	X:153735762	C	68	T	4	A149T

Table 2. Single nucleotide variations and indels detected by whole transcriptome sequencing. Only the genes with a variant allele frequency (Vaf) of ≥0.30 are included.

Gene	Position	Reference	Alternative	Total depth	Total Vaf	Amino acid change
FMNL1	17:43318777	GGC	GCG	1,125	0.9724	GP454GA
KIT	4:55592103	G	T	44	0.5909	S476I
AGBL5	2:27276292	C	T	21	0.5714	R80W
IPO4	14:24649767	C	T	58	0.5172	A1043T
CLIP1	12:122794329	C	T	49	0.4898	E1192K
XAB2	19:7685290	G	A	209	0.4545	R713W
KDM5B	1:202705466	G	A	29	0.4483	R1047
CYP51A1	7:91743154	C	T	45	0.4222	R452H
IL7R	5:35874575	C	T	149	0.4027	T244I

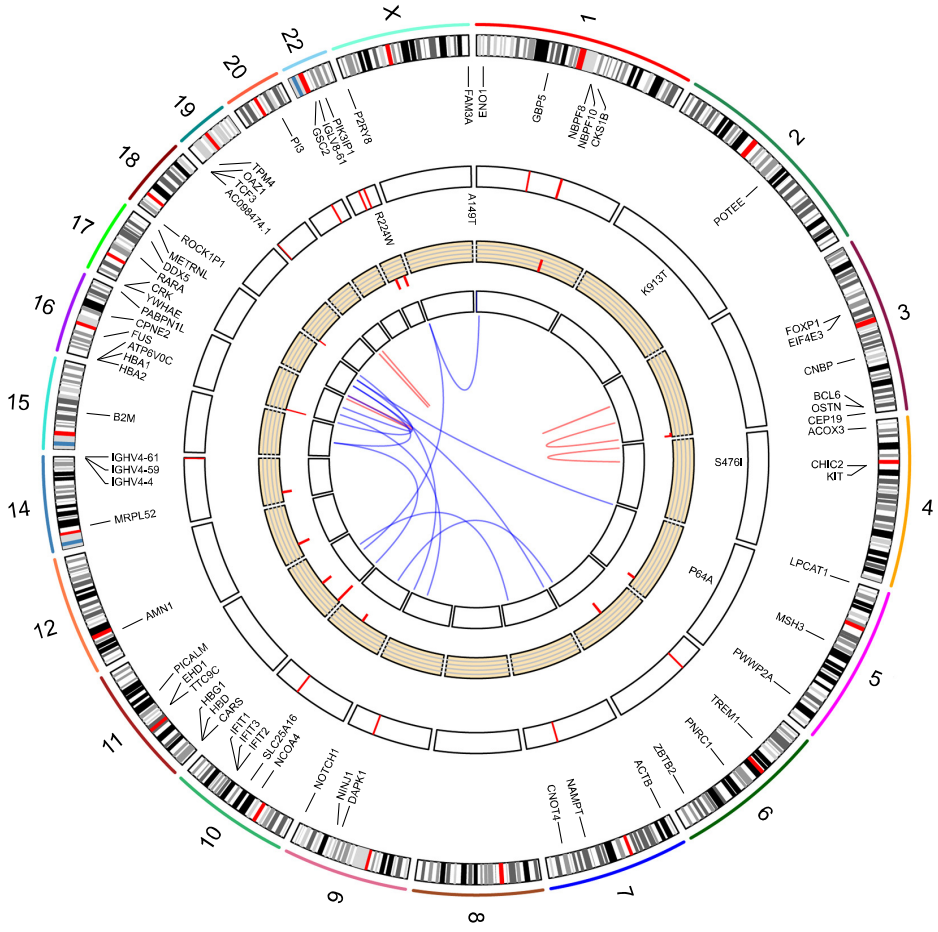

Fig. 1. Circus plot of structural variations, copy number variations, single nucleotide variations, and differentially expressed genes for mast cell leukemia. From the inner to outer track, this plot includes interchromosomal and intrachromosomal fusion genes (blue and orange), copy number variations (centripetal red line for loss, centrifugal red line for gain), genotype of single nucleotide variations, remarkably expressed genes (red line, RPKM ≥25), gene names, and chromosomal numbers. Chromosomes without mutations are not shown.

pression analysis with the WTS data, we observed an upregulation of the PI3K/AKT pathway, which is downstream of KIT and the mammalian target of rapamycin (mTOR). Fig. 1 presents a circus plot of the genetic profile of MCL in this patient. The list of genes in the circus plot can be found in Table 1 and Tables 3–5.

Based on our WES and WTS results, we first administered treatment with all-trans retinoic acid (ATRA), which targets RARα. After 2 weeks of ATRA, the patient's left eye protruded. An orbital CT scan showed the presence of a retro-orbital mass lesion, probably chloroma. Because ATRA failed to demonstrate efficacy, the patient was treated with dasatinib, which targets KIT. Her leg and abdominal pain improved transiently, but worsened after one month. Suspecting disease progression, we stopped dasatinib treatment and began treatment with everolimus, which targets the mTOR pathway. At this time, we also requested a PI3K inhibitor from a pharmaceutical company. Two weeks after beginning everolimus treatment, neutropenia and thrombocytopenia became prominent, probably related to adverse effects of the drug. Despite the everolimus treatment, the patient's bone pain did not improve. Unfortunately, a sudden cardiac arrest occurred 4 weeks later while waiting for the PI3K inhibitor as an alternative drug. She survived 11 months

Table 3. Differentially expressed gene list described in the circus plot of **Fig. 1**.

Gene	Chromosome	Start	End	RPKM
GBP5	1	89724632	89738544	62.108
NBPF10	1	145289771	145370303	52.277
NBPF8	1	144146807	144224481	33.603
TREM1	6	41235663	41254457	26.727
NAMPT	7	105888730	105926772	102.615
NINJ1	9	95883770	95896570	40.787
IFIT1	10	91152302	91163745	29.847
IFIT2	10	91061711	91069033	85.467
IFIT3	10	91087650	91100728	46.8
IGHV4-4	14	106478109	106478603	69.091
IGHV4-59	14	107081805	107083830	67.259
IGHV4-61	14	107095125	107095662	133.311
AC098474.1	19	229639	230165	34.455
PI3	20	43803516	43805185	66.326
IGLV8-61	22	22453109	22453622	317.57
PIK3IP1	22	31677578	31688520	27.04

Table 4. Gene list of copy number variations described in the circus plot of **Fig. 1**.

Gene	Chromosome	Start	End	Log_2 ratio
CKS1B	1	154947190	154951286	0.612
OSTN	3	190930312	190967927	−0.108
CEP19	3	196434362	196438871	−0.354
PWWP2A	5	159505108	159546423	−0.391
PNRC1	6	89790583	89793933	−0.611
SLC25A16	10	70243146	70287093	−0.414
HBD	11	5253852	5255677	−0.846
HBG1	11	5269532	5271134	−1.387
TTC9C	11	62495781	62505866	−0.538
AMN1	12	31825214	31862307	−0.59
MRPL52	14	23299075	23303575	−0.468
HBA2	16	222898	223654	−1.752
HBA1	16	226702	227465	−2.863
ROCK1P1	18	112377	120792	−0.389
GSC2	22	19136435	19137744	−0.531
RBX1	22	41347351	41368545	−0.542

Table 5. Fusion gene list described in the circus plot of **Fig. 1**.

5´ Gene	Chromosome	Start	End	3´ Gene	Chromosome	Start	End
CARS	chr11	3022152	3078681	*B2M*	chr15	45003685	45010357
FUS	chr16	31191431	31206192	*DAPK1*	chr9	90112143	90323549
CBFA2T3	chr16	88941263	89043504	*PABPN1L*	chr16	88929748	88933068
YWHAE	chr17	1247834	1303556	*CRK*	chr17	1324647	1359561
RARA	chr17	38465423	38513895	*B2M*	chr15	45003685	45010357
DDX5	chr17	62494374	62502484	*ATP6V0C*	chr16	2563727	2570224
YWHAE	chr17	1247834	1303556	*CPNE2*	chr16	57126455	57181878
TPM4	chr19	16178317	16213813	*KLF2*	chr19	16435651	16438339
OAZ1	chr19	2269485	2273487	*TCF3*	chr19	1609289	1650286
EIF4E3	chr3	71728440	71778269	*FOXP1*	chr3	71003865	71632904
CNBP	chr3	128886658	128902810	*BCL6*	chr3	187439165	187454285
CHIC2	chr4	54875958	54930815	*ACOX3*	chr4	8368009	8442452
LPCAT1	chr5	1461542	1524076	*DDX5*	chr17	62494374	62502484
ZBTB2	chr6	151685250	151712835	*NCOA4*	chr10	51565108	51590734
ACTB	chr7	5566779	5570232	*RARA*	chr17	38465423	38513895
CNOT4	chr7	135046547	135194875	*PICALM*	chr11	85668214	85780139
NOTCH1	chr9	139388896	139440238	*METRNL*	chr17	81037567	81052871
FAM3A	chrX	1581466	1656037	*ENO1*	chr1	8921059	8939151
FAM3A	chrX	1581466	1656037	*EHD1*	chr11	64620199	64647185

after the diagnosis of MCL.

DISCUSSION

Before our current study, only one WES study in a patient with MCL patient had been published in 2012 [10]. This study detected a point mutation in IgE mast-cell receptor β chain and *KIT*, but the treatment approach was not reported. With the exception of that WES study, most other MCL studies have focused on *KIT* mutations. *KIT* D816V mutation was detected in more than 90% of patients with an SM. Almost 40% of de novo patients with MCL had the *KIT* D816V mutation [1]. In addition, S476I, F522C, V654A, V560G, duplication of amino acids 501–502 and 502–503, and deletion of amino acids 501–502 were also reported in a case report [10-15]. Besides the *KIT* mutation, a *TET2* mutation has been investigated in aggressive SM [16]. Few patients with MCL had a 5q deletion, but all of those patients had secondary MCL that evolved from myelodysplastic syndrome [17].

Notably, *the KIT* mutation is the most important and prevalent pro-oncogenic mutation in MCL. In this study, we found the presence of a *KIT* S476I mutation, which has been discovered previously in chronic MCL [15]. With our finding, this point mutation becomes the second most common point mutation in MCL, following *KIT* D816V, which is located in the tyrosine kinase domain. *KIT* S476I, on the other hand, is located in the immunoglobulin-like extracellular domain, the function of which is not yet known [18]. A small portion of patients with MCL show improvement upon treatment with KIT inhibitors, such as imatinib or dasatinib, but dasatinib was not effective in our patient. Because a few reports showed promising results of PKC412 (midostaurin) for aggressive SM and MCL, we considered administering PKC412 to the patient [19-21]. However, the drug was not available at that time in Korea. Recently, selective inhibitors targeting specific *KIT* mutations, such as *KIT* D816V, have been studied [22], and selective drugs that block *KIT* S476I could be beneficial to a proportion of MCL patients. Because no selective KIT inhibitor for S476I is approved currently, we blocked pathways downstream of *KIT*. One of the downstream pathways is the PI3K/AKT/mTOR pathway, which was upregulated in this MCL case. However, treatment with an mTOR inhibitor regrettably was not effective in this patient.

Several plausible explanations could account for the treatment failure. One explanation would be targeted therapy alone. In patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (ALL), BCR-ABL tyrosine kinase inhibitor (TKI) was used as an induction treatment combined with cytotoxic multiagent chemotherapy, although BCR-ABL TKI alone was very effective in chronic myeloid leukemia [23]. Like ALL, acute MCL, one form of acute leukemia, might be too aggressive to treat with TKI alone. Another explanation could be that *KIT* S476I, the PI3K/AKT/mTOR pathway, or RARα fusion protein was not a main driver mutation or pathway in this MCL pathogenesis. Although the above variations were detected in sequencing data, our analysis did not exclude the possibility that they were only passenger mutations. Finally, it is possible that the drug dosage level was insufficient, given that the optimal dosage of TKI agents has not been determined for acute MCL.

In conclusion, we presented a case study of a patient with an orphan disease in which we used a targeted approach to therapy with WES and WTS data from the patient. Although this approach did not successfully cure the disease, she survived 11 months, approximately twice as long as the median survival of patients with acute MCL. The results of our treatments were not ideal, but the utility of this type of approach should be further researched and validated in the future.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Georgin-Lavialle S, Lhermitte L, Dubreuil P, Chandesris MO, Hermine O, Damaj G. Mast cell leukemia. Blood 2013;121:1285-95.
2. Ustun C, Reiter A, Scott BL, et al. Hematopoietic stem-cell transplantation for advanced systemic mastocytosis. J Clin Oncol 2014;32:3264-74.
3. Valent P, Sotlar K, Sperr WR, et al. Refined diagnostic criteria and classification of mast cell leukemia (MCL) and myelomastocytic leukemia (MML): a consensus proposal. Ann Oncol 2014;25:1691-700.
4. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25:1754-60.
5. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010;20:1297-303.
6. Cibulskis K, Lawrence MS, Carter SL, et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol 2013;31:213-9.
7. Li J, Lupat R, Amarasinghe KC, et al. CONTRA: copy number analysis for targeted resequencing. Bioinformatics 2012;28:1307-13.
8. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078-9.
9. Ge H, Liu K, Juan T, Fang F, Newman M, Hoeck W. FusionMap: detecting fusion genes from next-generation sequencing data at base-pair resolution. Bioinformatics 2011;27:1922-8.
10. Spector MS, Iossifov I, Kritharis A, et al. Mast-cell leukemia exome sequencing reveals a mutation in the IgE mast-cell receptor β chain and KIT V654A. Leukemia 2012;26:1422-5.
11. Akin C, Metcalfe DD. Systemic mastocytosis. Annu Rev Med 2004;55:419-32.
12. Georgin-Lavialle S, Aguilar C, Guieze R, et al. Mast cell sarcoma: a rare and aggressive entity-report of two cases and review of the

literature. J Clin Oncol 2013;31:e90-7.
13. Mital A, Piskorz A, Lewandowski K, Waśąg B, Limon J, Hellmann A. A case of mast cell leukaemia with exon 9 KIT mutation and good response to imatinib. Eur J Haematol 2011;86:531-5.
14. Georgin-Lavialle S, Lhermitte L, Suarez F, et al. Mast cell leukemia: identification of a new c-Kit mutation, dup(501-502), and response to masitinib, a c-Kit tyrosine kinase inhibitor. Eur J Haematol 2012;89:47-52.
15. Valent P, Berger J, Cerny-Reiterer S, et al. Chronic mast cell leukemia (MCL) with KIT S476I: a rare entity defined by leukemic expansion of mature mast cells and absence of organ damage. Ann Hematol 2015;94:223-31.
16. Soucie E, Hanssens K, Mercher T, et al. In aggressive forms of mastocytosis, TET2 loss cooperates with c-KITD816V to transform mast cells. Blood 2012;120:4846-9.
17. Valentini CG, Rondoni M, Pogliani EM, et al. Mast cell leukemia: a report of ten cases. Ann Hematol 2008;87:505-8.
18. Lennartsson J, Rönnstrand L. Stem cell factor receptor/c-Kit: from basic science to clinical implications. Physiol Rev 2012;92:1619-49.
19. Gotlib J, Berubé C, Growney JD, et al. Activity of the tyrosine kinase inhibitor PKC412 in a patient with mast cell leukemia with the D816V KIT mutation. Blood 2005;106:2865-70.
20. Growney JD, Clark JJ, Adelsperger J, et al. Activation mutations of human c-KIT resistant to imatinib mesylate are sensitive to the tyrosine kinase inhibitor PKC412. Blood 2005;106:721-4.
21. Quintas-Cardama A, Aribi A, Cortes J, Giles FJ, Kantarjian H, Verstovsek S. Novel approaches in the treatment of systemic mastocytosis. Cancer 2006;107:1429-39.
22. Lee S, Lee H, Kim J, et al. Development and biological evaluation of potent and selective c-KIT(D816V) inhibitors. J Med Chem 2014;57:6428-43.
23. Kim DY, Joo YD, Lim SN, et al. Nilotinib combined with multiagent chemotherapy for newly diagnosed Philadelphia-positive acute lymphoblastic leukemia. Blood 2015;126:746-56.