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Potent efficacy of chlorpromazine in acute myeloid leukemia harboring *KIT-D816V* mutation

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ABSTRACT

Acute myeloid leukemia (AML) is a heterogeneous disease often associated with poor prognosis. We previously showed that the localization of KIT-D816V at endolysosomes is critical to activate aberrant Akt signaling and Chlorpromazine (CPZ) perturbs the intracellular localization, leading to cell death in AML cells with *KIT-D816V*. We report that daily administration of CPZ, prescribed for controlling anxiety disorder in patient with AML harboring *KIT-D816V*, led to a dramatic reduction in AML cells. *In vitro* and *in vivo* experiments showed that CPZ inhibited the growth and survival of the patient-derived AML cells, implying potent efficacy of CPZ in AML with *KIT-D816V*.

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease, and several genes are recurrently mutated in AML, which constitute the base for the new genomic classifications, predictive biomarkers, and new therapeutic targets. Despite the enormous progress achieved in the understandings of the disease, the standard therapy of AML has remained virtually unchanged for the past several decades.

KIT is a receptor tyrosine kinase (RTKs) which is expressed on the surface of hematopoietic stem/progenitor cells. A point mutation in the *KIT* gene at amino acid 816 (*KIT-D816V*) is an activating mutation that is found in about 30% of patients in core-binding factor (CBF)–AML [1]. In addition, the same mutation is typical of systemic mastocytosis, a rare disorder characterized by overproduction of mast cells that accumulate in the skin and organs. Although AML-M2 (according to the FAB classification) accompanied by the chromosomal translocation t(8;21)(q22; q22), in the following denoted as *RUNX1-RUNX1T1*, is classified into a good prognostic group, *KIT* exon 17 mutation is a poor prognostic factor in AML patients with *RUNX1-RUNX1T1*[2].

Wild-type (WT)-KIT plays an important role in the proliferation and maintenance of normal cells. Upon ligand binding, WT-KIT is internalized and transferred to early endosomes and after delivering proper signals to downstream molecules, a fraction of WT-KIT is transferred via late endosomes for its degradation in lysosomes. Notably, the intracellular trafficking of KIT-D816V is different and it may persist in intracellular compartments [3], leading to the sustained and aberrant oncogenic KIT signaling in endolysosomes and endoplasmic reticulum, which result in the uncontrolled proliferation of malignant cells [4].

We recently showed that Chlorpromazine (CPZ), which is a widely used as an antipsychotic drug, interferes with the intracellular localization of KIT-D816V, leading to cell death in AML cells with *KIT-D816V in vitro* and *in vivo*[5]. We herein report that daily administration of CPZ, prescribed for controlling anxiety disorder in an AML patient with *RUNX1-RUNX1T1* harboring *KIT-D816V*, led to a dramatic reduction in AML cells.

2. Materials & methods

In vitro and *in vivo* experiments were performed as described before [5]. The sample was obtained after written informed consent following institutional guidelines of Kindai University Faculty of Medicine (Authorization Number: 24–017, −018) per Declaration of Helsinki principles. The animal experiment was conducted after getting the approval from the committee of animal experiments in our university

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3. Results

3.1. Clinical course

A 70-year female with end-stage thyroid cancer was referred to our hospital in 2016 because of the symptoms of weakness. Laboratory tests revealed a Hb level of 7.7 g/dL, a white blood cell count (WBC) of 4200/μl, and a platelet count of 102,000/μL. A bone marrow (BM) aspirate showed increased myeloblasts of 20.2%, which were positive for myeloperoxidase staining. She was eventually diagnosed as having AML with *RUNX1-RUNX1T1* harboring the *KIT-D816V* [1] (**Fig. 1a**). She had been diagnosed as having follicular thyroid cancer in 2006 and had received total thyroidectomy followed by radioactive iodine remnant ablation therapy. In 2016, thyroid cancer recurred with multiple pulmonary metastases and massive right iliac bone metastases, which gradually progressed thereafter. She was judged as not eligible for any antileukemic therapy due to her poor performance status, and thus only palliative care was offered. Twenty-one days after the diagnosis of AML, she was admitted to our hospital due to a rapid increase of AML blasts (WBC 15,000/μl, AML blasts 83.6%). CPZ was given to her to relieve insomnia refractory to standard sleep drugs and to ameliorate the symptoms of anxiety disorders. The starting dose of CPZ was 50 mg, qd at night, which was further increased up to 75 mg qd, leading to the successful control of the symptoms (**Fig. 1b**). Although grade 1 somnolence was observed, it was manageable without dose reduction of CPZ. Finally, CPZ did not show any other sign of side effect during the administration period. Fourteen days after starting CPZ, the number of AML blasts decreased drastically in peripheral blood (PB), and the patient was discharged on day 50 after the diagnosis of AML. The patient continued CPZ at 75 mg qd, and AML blasts were kept at a very low level in PB. However, she discontinued CPZ on day 70 after AML diagnosis because her anxiety disorder was resolved at that time. On day 84, she was readmitted to our hospital due to the deterioration of the symptoms caused by thyroid cancer metastasis. Laboratory tests revealed a concomitant recovery of the neutrophil count, however, the number of AML blasts slightly increased, suggesting imminent AML progression. Because of her symptoms of anxiety disorders, we restarted CPZ at the same dose, which again resulted in a reduction of AML blasts. However, she finally died of respiratory failure due to multiple lung metastases of thyroid cancer (**Fig. 1b**).

3.2. CPZ inhibits the growth/survival of AML cells with KIT-D816V in vitro and in vivo

Given the remarkable clinical improvement of the AML associated with CPZ administration, we assumed that the drug directly induced anti-leukemic effects in this patient. Therefore, in an attempt to obtain experimental evidence of the putative anti-leukemia effects of CPZ, we performed *in vitro* and *in vivo* experiments using AML cells isolated from the patient. At first, we performed annexin V staining using her AML cells after the culture with or without CPZ. As shown in **Fig. 2a**, CPZ treatment increased apoptotic cells detected as an Annexin V-positive fraction. Furthermore, we found that not only her AML cells but also the human (h)$CD34^{+}hCD38^{-}$ fraction, which contains AML initiating cells, were highly sensitive to CPZ *in vitro* (**Fig. 2b, c**). Next, we tested anti-leukemic activities of CPZ in a xenograft mouse model using her AML cells at the same CPZ concentrations utilized in our previous study [5]. For the first few days after transplantation, the CPZ treated mice slept for half a day, and slight weight loss was observed compared to the control mice, however, the side effect of weight loss was transient. The body weight was comparable between the two groups at eight weeks after transplantation (**Fig. 2d**), and the mice on the CPZ group did not show any sign of sickness prior to being euthanized. In control mice, mean percentage of $hCD45^{+}$ cells in the BM were 5.16% at eight weeks after transplantation, and these cells harbor *KIT-D816V* mutation, indicating engraftment of her AML cells. In contrast, CPZ drastically reduced AML cells in the CPZ treated mice (mean percentage of $hCD45^{+}$ cells; 1.46%, $p = 0.031$) (**Fig. 2e**). These results are consistent with the assumption that AML remission observed in this patient was very likely induced by CPZ.

4. Discussion

We here report a case in which CPZ, given for treating refractory insomnia and anxiety disorder, was effective for her AML with *KIT-D816V*. Discontinuation of CPZ led to AML relapse. However, CPZ was again effective for relapsed AML, whereas this patient consequently died due to lung metastasis of thyroid cancer.

AML with *RUNX1-RUNX1T1* is considered a distinct AML entity that associates with a good prognosis [1]. As for the prognostic impact of *KIT* mutation, however, controversial results have been reported [2]. Whereas some reports showed that *KIT* mutation was a poor prognostic factor in CBF–AML, other studies reported that the *KIT* mutation has no prognostic impact on CBF–AML [1,2]. Recently, the adverse effects of *KIT* mutation were observed only in AML patients with *RUNX1-RUNX1T1* in the large prospective study [2].

The development of tyrosine kinase inhibitors with clinical utility in AML is an area of active research. Since the imatinib is a KIT inhibitor, it has been tested in AML with or without *KIT-D816V*. Although it certainly induced partial clinical responses in subsets of AML patients treated within clinical trials, its effects were limited and thus currently, there is no promising KIT inhibitor that can be utilized for AML.

CPZ is a phenothiazine drug that has been utilized for many years to treat several psychiatric disorders. Pharmacologically, CPZ acts as an antagonist on different postsynaptic and presynaptic receptors, including dopamine, serotonin, and histamine 1 receptor, which accounts for its versatility and clinical utility in various disorders [5,6]. In addition, preclinical studies have reported that CPZ has antitumor activities in several cellular systems [5,7,8]. Furthermore, it has been reported that CPZ inhibits clathrin-mediated endocytosis through disruption of clathrin-coated pit formation by a reversible translocation of clathrin and its adapter proteins from the plasma membrane to intracellular vesicles and subsequently induces anti-tumor effects against various cancer cell lines [5,9].

Oncogenic *KIT-D816V* can cause aberrant signals not just from the plasma membrane, but also from intracellular compartments. It accumulates on endolysosomes through clathrin-mediated endocytosis from the cell surface, where it activates aberrant Akt signaling [4]. We recently showed that the localization of KIT-D816V at endolysosomes is critical to activate aberrant Akt signaling, and CPZ perturbs the intracellular localization of KIT-D816V at endolysosomes, thereby exhibiting anti-leukemic activities against AML cells with *KIT-D816V* [5]. Importantly, *in vitro* and *in vivo* experiments showed that CPZ effectively inhibited the growth and survival of her AML cells. There are some reports in which spontaneous remission of AML has been observed, particularly during the course of severe infection or after blood transfusion have been reported [10], they are extremely rare cases and may be in association with the emergence of a powerful immune response in the host that eradicates AML cells.

In conclusion, we have presented a case where CPZ was effective for AML with *KIT-D816V*. *In vitro* and *in vivo* studies showed that CPZ effectively inhibited the growth and survival of her AML cells. These results support the potent efficacy of CPZ in AML with *KIT-D816V*. We believe that testing the efficacy of CPZ in clinical trials with a large number of patients is warranted.

CRediT authorship contribution statement

Shinya Rai: . **Hirokazu Tanaka:** . **J. Luis Espinoza:** . **Takahiro Kumode:** Data curtion. **Itaru Matsumura:** Writing – review & editing.

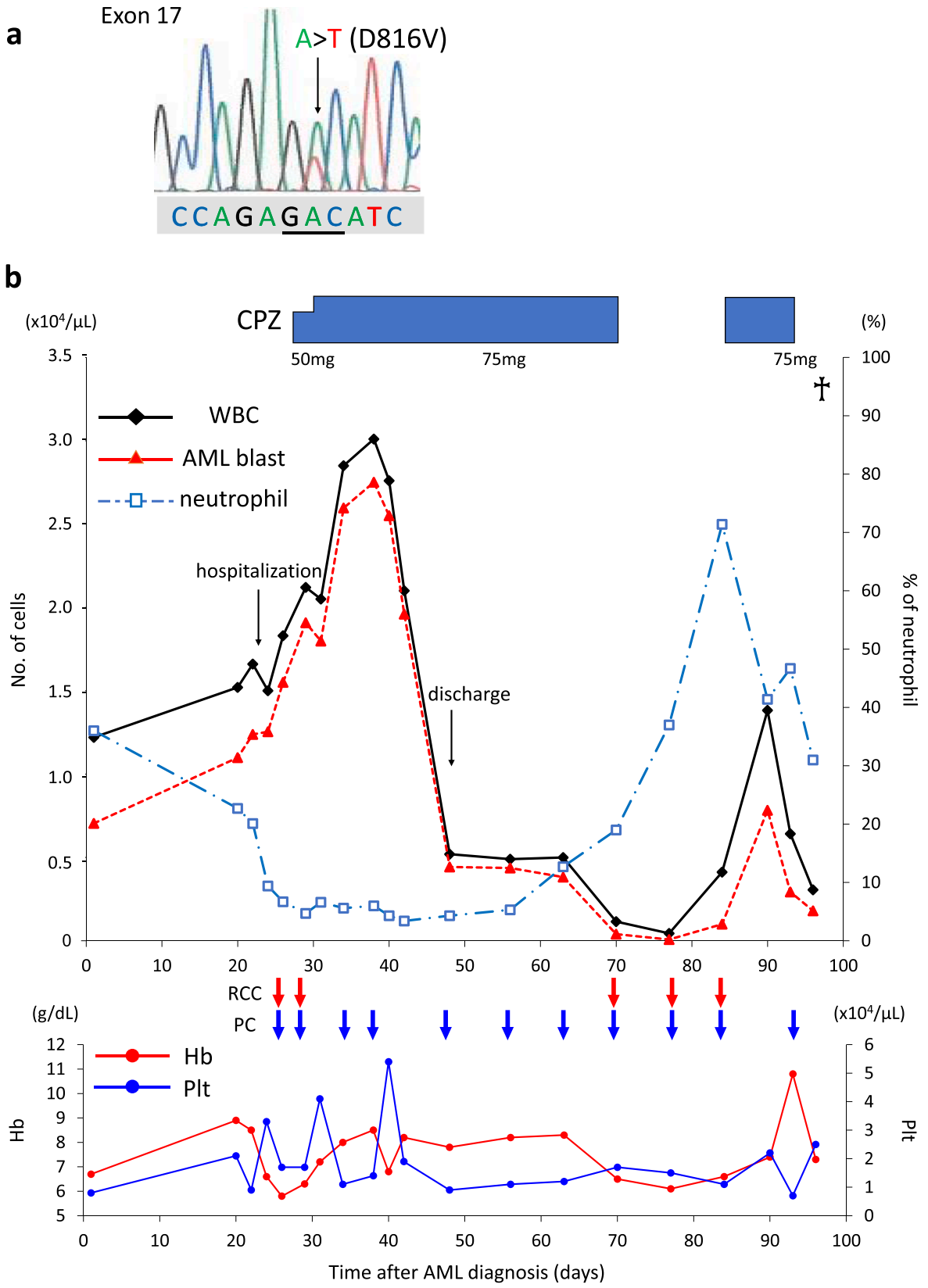

Fig. 1. The clinical course of an AML patient with *KIT-D816V*, who received CPZ. (a) Genomic DNA was extracted using a Wizard genomic DNA purification kit (Promega), and human KIT gene was analyzed by Sanger sequencing. The sequence in AML cells with *KIT-D816V* mutation is shown. (b) Changes in total number of white blood cells (WBC) and AML blasts, the percentage of neutrophils (upper panel), and the levels of hemoglobin (Hb) and platelet (Plt) (lower panel) in the peripheral blood are shown. Relevant time points such as diagnosis, hospitalization and discharge are indicated by arrows. CPZ was performed during the indicated periods. Transfusion of red cell concentrate (RCC) and platelet concentrate (PC) was performed as indicated in clinical practice.

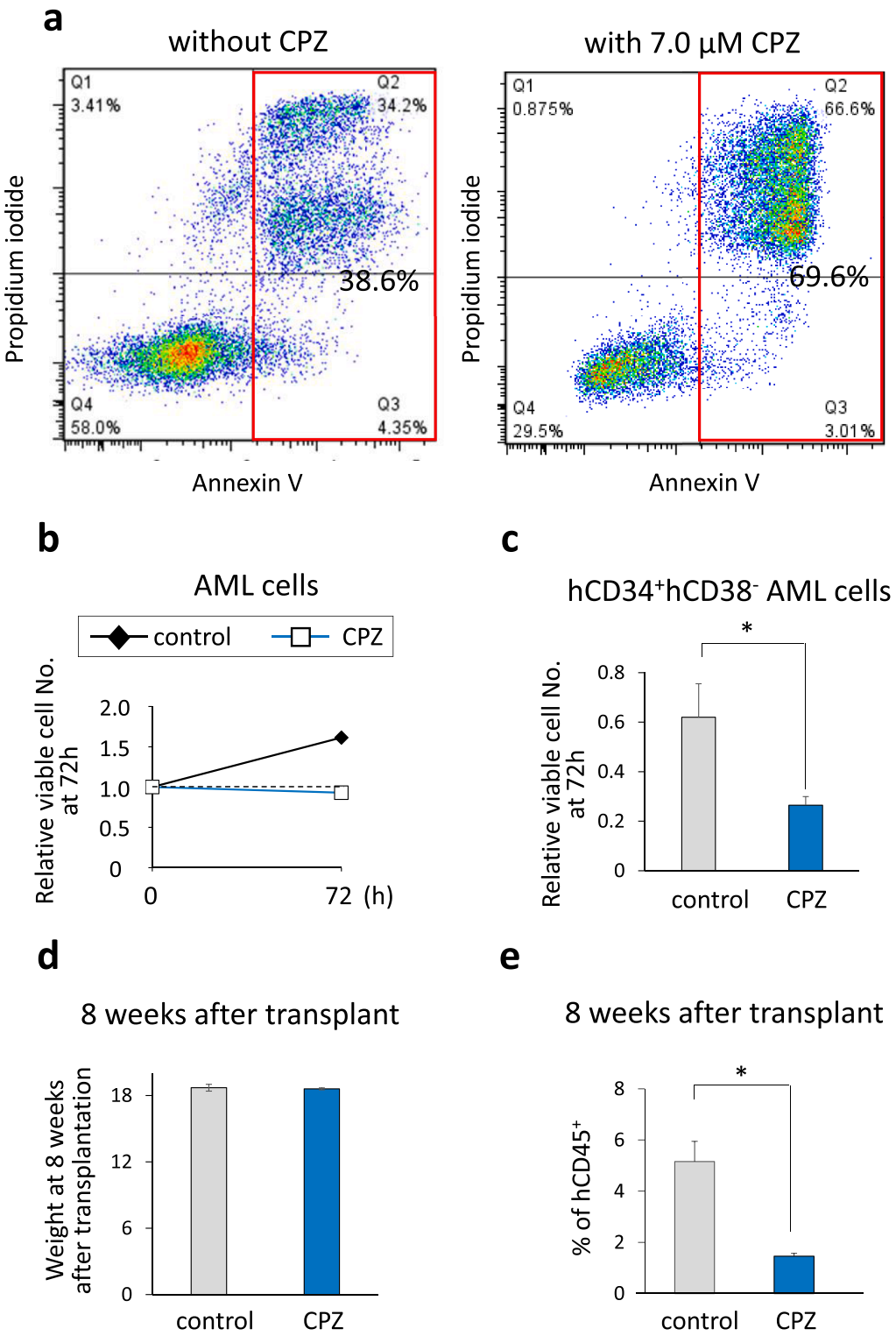

Fig. 2. Experimental evidence of the anti-leukemia effects of CPZ *in vitro* and *in vivo*. (a) Bone marrow mononuclear cells (BMMNCs) were isolated from the patient. These cells were cultured in the presence of SCF, FL, and TPO with 7.0 μM CPZ (right panel) or without CPZ (left panel) for 72 h, and then apoptotic cells were detected as Annexin V-positive cells by flow cytometry. (b) BMMNCs were cultured in the presence of SCF, FL, and TPO with or without 7.0 μM CPZ for 72 h, and then cell growth was quantified. Relative viable cell numbers after CPZ treatment were calculated using untreated cells as a reference. (c) $hCD34^{+}hCD38^{-}$AML cells were isolated from BMMNCs of the patient and cultured in the presence of SCF, FL, and TPO with or without 7.0 μM CPZ for 72 h and then cell growth was quantified. The results indicate the standard error of the mean (SEM) from three independent experiments. *$p<0.05$, Student's *t*-test. (d) Eight weeks after transplantation, the body weight was compared between the two groups. The results indicate the mean ± SEM from three independent experiments. (e) After transplantation of primary AML cells from the patient, mice were treated with CPZ or normal saline (as a control). Eight weeks after transplantation, the proportion of $hCD45^{+}$ cells in the BM was assessed by flow cytometry. Figures depict the mean ± SEM of the%$hCD45^{+}$ cells in each group ($n = 3$). *$p<0.05$, Student's *t*-test.

Declaration of Competing Interest

Itaru Matsumura Honoraria: Astellas, Novartis, Shionogi Pharmaceuticals No other potential conflicts of interest were reported.

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