Induction of Effector and Memory Cellular Immunity in a Patient with Long-Term Complete Molecular Response to Philadelphia Chromosome-Positive Acute Lymphoblastic Leukemia

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Abstract
This case report is about a patient who suffered from Philadelphia chromosome (Ph1)-positive acute lymphoblastic leukemia. The blasts were positive for myeloid-lineage markers including CD13 and CD33, as well as B-cell-lineage markers. Minor bcr-abl1 mRNA was detected by real-time quantitative polymerase chain reaction. Chromosomal abnormality monosomy 7 was also observed, in addition to Ph1. Despite treatment difficulties that were anticipated based on these findings, the patient had long-time complete molecular response (CMR) for approximately 5 years using chemotherapy and two tyrosine kinase inhibitors, imatinib and dasatinib. Lymphocytes were elevated after the patient switched from imatinib to dasatinib, and a T-cell receptor (TCR) V beta gene repertoire analysis revealed oligoclonal expansion of effector and memory cytotoxic T lymphocytes (CTLs), including Wilms tumor 1-specific CTLs. More specifically, the two memory CTLs expressing TCR V beta 3 and V beta 7.1 gradually increased after dasatinib administration. The activation and maintenance of anti-leukemia immunity may have allowed the patient to obtain long-time CMR. These results highlight that obtaining memory CTLs for leukemia cells may lead to safe withdrawal from dasatinib in the patient.

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Introduction

Philadelphia chromosome (Ph1), resulting from the reciprocal translocation between chromosomes 9 and 22, can lead to the formation of major and minor $bcr-abl_1$ chimeric genes [1]. Major $bcr-abl_1$ is generally detected in chronic myeloid leukemia (CML), whereas minor $bcr-abl_1$ is usually detected in Ph1+ acute lymphoblastic leukemia (ALL), a chemotherapy-refractory hematologic neoplasm [1]. Dasatinib, a second-generation tyrosine kinase inhibitor (TKI) that blocks multiple tyrosine kinases, such as BCR-ABL and Src kinases, is approved for CML and Ph1+ ALL [2]. Patients treated with dasatinib, but not other TKIs, often experience elevated lymphocyte counts and large granular lymphocytes [3, 4]. This case report is of a patient with Ph1+ ALL who achieved long-term survival. The patient developed oligoclonal effector and memory cytotoxic T lymphocytes (CTLs), including Wilms tumor 1 (WT1)-specific CTLs.

Case Presentation

A 54-year-old female was admitted to our hospital with leukocytosis and abnormal cells. Table 1 summarizes the laboratory data. The patient had an elevated leukocyte count (28,400/µL) with 75.5% blasts; and slight thrombocytopenia was also observed (11.9 × 10⁴/µL). Bone marrow aspiration results were not available due to a dry tap. Flow cytometry of the peripheral mononuclear cells revealed that the blasts were positive for CD10, CD13, CD19, CD33, and CD34 and negative for CD2, CD3, CD4, CD5, CD7, CD8, CD14, CD20, CD41, CD56, and CD235a. Ph1 positivity with monosomy 7 was detected by the chromosomal analysis of peripheral blood. Messenger RNA, extracted from peripheral mononuclear cells and analyzed by real-time quantitative polymerase chain reaction (RQ-PCR), revealed that the patient was positive for minor $bcr-abl_1$ and negative for major $bcr-abl_1$. Based on this data, the patient was diagnosed with Ph1+ ALL. Induction chemotherapy, comprised of daunorubicin (50 mg/m² on days 1–5) and cytarabine (100 mg/m² on days 1–7), was initiated. One month later, dasatinib (140 mg/day) was added after the identification of minor $bcr-abl_1$. Complete remission was confirmed 1 month after the induction therapy, and complete molecular response (CMR, no detectable minor $bcr-abl_1$ by RQ-PCR) was observed 2 months after the induction therapy. Therefore, the first consolidation therapy, comprised of daunorubicin (45 mg/m² on days 1–3), vincristine (2 mg/body on days 1 and 8), and prednisolone (60 mg/m² on days 1–7), was administered 1 month after induction therapy (consolidation A). The patient has maintained complete remission and CMR to date. Thereafter, a second consolidation therapy (consolidation B) round, comprised of etoposide (150 mg/m² on days 1–4) and cytarabine (2,000 mg/m² on days 1–4), was administered 2 months after induction therapy, and the dasatinib dose was reduced to 100 mg/day 3 months after the induction therapy. The third consolidation therapy (consolidation C) round, comprised of methotrexate (2,360 mg/m² on days 1 and 15), was administered 3 months after induction therapy. One cycle of consolidation B and one cycle of consolidation C were administered 4 and 5 months after induction therapy, respectively. Thereafter, the patient has had outpatient follow-ups.

Dasatinib was changed to the first-generation TKI imatinib (600 mg/day) 7 months after initiating therapy, due to a pleural effusion that was considered a dasatinib-associated adverse event (Fig. 1A). The imatinib dose was reduced to 400 mg/day 9 months after initiating therapy and continued for 23 months, when it was changed to a reduced-dose dasatinib (20 mg/day initially and 40 mg/day 36 months after initiating therapy) in combination with diuretics per the patient’s preference. After the switch to dasatinib, the lymphocyte counts gradually increased (Fig. 1B) [3, 4]. The treatments are summarized in further detail in Figure 1.
T-cell receptor (TCR) Vβ repertoire analysis by flow cytometry using a beta mark TCR Vβ repertoire kit (Beckman Coulter, Tokyo, Japan) was conducted 31 months after the induction therapy. Figure 2 highlights the changes in the rates of effector and memory CTLs. Our analyses revealed two effector CTL clones, expressing TCR Vβ 3 and Vβ 17 genes, and two memory CTL clones, expressing TCR Vβ 3 and Vβ 7.1 genes. More specifically, the two memory CTL clones gradually increased after resuming dasatinib administration (Fig. 1 and 2). The patient harbored human leukocyte antigens (HLAs)-A*02:06:01 and -A*31:01:02. Some CML-specific tumor antigens, restricted by HLA-A*02:01/A*02:06:01, were also previously reported [5, 6]. Therefore, a tetramer analysis was performed on the patient’s peripheral blood. All tetramers were purchased from BML (Nagoya, Japan). Cytomegalovirus-specific CTLs and CTLs recognizing WT137–45 were detected 56 months after the induction therapy (Table 2).

| Table 1. Laboratory data on first admission |
|-------------------------------------------|
| **Complete blood counts**                 |
| White blood cells                         | 28,400/μL |
| Blasts                                    | 75.5%     |
| Myelocytes                                | 0.5%      |
| Segmented neutrophils                     | 8.5%      |
| Lymphocytes                               | 12.5%     |
| Atypical lymphocytes                      | 1.0%      |
| Monocytes                                 | 1.5%      |
| Red blood cells                           | 435×10⁴/μL|
| Hemoglobin                                | 13.6 g/dL |
| Hematocrit                                | 39.4%     |
| Platelet                                  | 11.9×10⁴/μL|
| **Bone marrow aspiration**                |
|                                          |
| **Flow cytometry (peripheral mononuclear cells)** |
| T/NK-cell lineage                         |
| CD2: 0.1%                                 |
| CD3: 0.1%                                 |
| CD4: 0.1%                                 |
| CD5: 0.1%                                 |
| CD7: 3.5%                                 |
| CD8: 0.0%                                 |
| CD56: 0.0%                                |
| B-cell lineage                            |
| CD10: 97.7%                               |
| CD19: 99.9%                               |
| CD20: 0.9%                                |
| Myeloid lineage/others                    |
| CD13: 99.6%                               |
| CD14: 0.2%                                |
| CD33: 82.7%                               |
| CD34: 99.8%                               |
| CD41: 2.4%                                |
| CD117: 18.0%                              |
| CD235a: 1.6%                              |
| HLA-DR: 18.0%                             |
| Chromosomal analysis (peripheral blood)   |
| 45,XX,−7,t(9;22)[4]/46,XX[1]              |
| bcr-abl1 mRNA analysis (RQ-PCR)           |
| Major bcr-abl1 mRNA                       |
| Not detected                              |
| Minor bcr-abl1 mRNA                       |
| 1.3×10⁵ copies/μg RNA                     |
| RQ-PCR, real-time quantitative polymerase chain reaction. |
Discussion/Conclusion

First- and second-generation TKIs have led to significant improvements in treating both CML and Ph1+ ALL. Patients with chronic phase (CP)-CML who attain deep molecular responses will have a life expectancy comparable to that of the general population [7]. However, TKIs alone cannot eradicate Ph1+ cancer stem cells [8]. Discontinuing TKI therapy to obtain...
Fig. 2. T-cell receptor V beta gene repertoire analysis. **A** Effector cytotoxic T lymphocytes (CTLs) (%). **B** Memory CTLs (%). Peripheral blood mononuclear cells were analyzed by flow cytometric analysis using the beta mark TCR Vβ repertoire kit (Beckman Coulter, Tokyo, Japan) according to the manufacturer’s instructions. Effector CTLs were defined as the CD8+CD27-CD45RA + population, and memory CTLs were defined as the CD8+CD45RA-population.

| Tumor antigen | Peptides     | Cytotoxic T lymphocytes (%) |
|---------------|--------------|----------------------------|
| CMV           | NLVPMVATV    | 2.012                      |
| WT137–45      | VLDFAAPGA    | 0.006                      |
| WT1226–134    | RMFPNAPYL    | 0.000                      |
| hTERT         | ILAKFLHVL    | 0.000                      |
| BCR-ABL       | GVRGRVEEI    | 0.000                      |
| PR1           | VLQELNVT    | 0.000                      |

The patient’s peripheral blood was directly analyzed by tetramer analysis to detect tumor antigen-specific CTLs, in the absence of stimulation. All tetramers were purchased from BML (Nagoya, Japan). CML, chronic myeloid leukemia; WT1, Wilms tumor 1; hTERT, human telomerase reverse transcriptase.

treatment-free remission (TFR) has been investigated previously in various conditions; half of all patients can maintain a deep molecular response [9]. Considering the immunological aspects related to the efficacy of interferon alpha, hematopoietic stem cell transplantation, and donor leukocyte infusion, upregulating cellular immunity in Ph1+ cancer stem cells may be deemed necessary to obtain a cure. We have recently reported on a patient with CP-CML who maintained TFR for several years after the cessation of dasatinib [10]. Maintenance of memory and effector CTLs with TCR clonality observed in the previous patient suggested that clonal expansion of CTLs against CML stem cells may be a meaningful marker of TKI cessation [10].
The blasts of the present patient expressed myeloid-lineage markers CD13 and CD33, as well as B-cell-lineage markers. Furthermore, the blasts harbored monosomy 7 in addition to Ph1. Although difficulties in treatment were anticipated based on these findings, the patient was able to achieve long-term CMR for approximately 5 years, using chemotherapy and TKI treatment. Cytotoxic cellular immunity, assessed by TCR \( V \beta \) gene repertoire analysis, revealed the oligoclonal expansion of effector and memory CTLs (Fig. 2). Interestingly, two memory CTL clones expressing TCR \( V \beta 7.1 \) and \( V \beta 3 \) gradually increased after the resumption of dasatinib 32 months after initiation therapy. The data suggests that dasatinib may play two roles: acting as a molecular-targeted therapy and activating and maintaining host cellular immunity against Ph1+ cancer stem cells. Based on our experience in a patient with CP-CML, as noted above, it may be safe to discontinue dasatinib in the present patient.

The analysis of several known tumor antigens in CML, using tetramer analysis, revealed that the present patient had WT137–45-specific CTLs. Tetramers for cytomegalovirus-specific CTLs were used as a positive control.

In summary, this case report describes a patient with Ph1 ALL, who was treated with chemotherapy and TKI treatment, and obtained long-term CMR. Dasatinib may have played an important role in activating host cellular immunity and maintaining effector and memory CTLs. These findings, coupled with our previous experience with a patient with CP-CML who achieved TFR, suggest that elevation in effector and memory CTL numbers against Ph1+ cancer stem cells may lead to long-term CMR and TFR.

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Statement of Ethics

The patient gave us written informed consent to publish the content of this manuscript including the use and disclosure of protected health information.

Disclosure Statement

The authors have no conflicts of interest to declare for this study.

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Author Contributions

T.J. wrote the manuscript including designing the work and acquiring, analyzing, and interpreting the data. Y.K., K.M., H.S., T.S., H.T., and S.H. conducted the acquisition and the analysis of data. M.M., and J.T. discussed the conception of the manuscript.
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