Arsenic induced complete remission in a refractory T-ALL patient with a distinct T-cell clonal evolution without molecular complete remission: A case report

SUIJING WU1*, LING XU2*, XIN HUANG1, SUXIA GENG1, YAN XU2,3, SHAOHUA CHEN2, LIJIAN YANG2, XIULI WU2, JANYU WENG1, XIN DU1 and YANGQIU LI2,3.

1Department of Hematology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong 510080; 2Institute of Hematology, Jinan University; 3Key Laboratory for Regenerative Medicine, Ministry of Education, Jinan University, Guangzhou, Guangdong 510632, P.R. China

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Abstract. Currently, arsenic trioxide therapy is widely used for the treatment of acute promyelocytic leukemia (APL), relapsed and refractory adult T-cell leukemia/lymphoma and myelodysplastic syndrome. Regarding the broad antitumor activity of arsenic, certain studies have been undertaken to test its efficacy in treating acute T-cell lymphoblastic leukemia (T-ALL) cell lines and patients; however, to the best of our knowledge, no reports document that arsenic is able to induce the remission of T-ALL patients. The present study reports the case of a young male patient diagnosed with T-ALL, with no significant response to common chemotherapy regimens, who finally achieved complete remission without minimal residual disease (as detected by flow cytometry) due to arsenic treatment. This result is encouraging, and the present study has shown that malignant TCRαβ+ cell clones can be detected at the molecular level using reverse transcription-polymerase chain reaction (PCR) combined with the GeneScan technique. The result is mainly based on the T-cell receptor (TCR) Vβ1 clone (a 190-base pair PCR product that with the same complementarity determining region 3 length can be detected for all samples collected during various statuses) and on undetectable TCR Vγ subfamily members, at the time of disease diagnosis. It is important to analyze the dynamically changing TCR pool in leukemia patients during therapy. Although the molecular mechanism through which arsenic contributes to malignant clone elimination remains unclear in the case presented, the use of arsenic is expected to be effective for clinically treating refractory and relapsed T-ALL patients.

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) comprises aggressive hematological tumors that derive from the malignant transformation of T-cell progenitors. T-ALL accounts for 10-15% of pediatric and 25% of adult acute lymphoblastic leukemia (ALL) cases (1). With the introduction of improved treatment regimens, including risk-adapted chemotherapy, hematopoietic stem cell transplantation (HSCT) and supportive care, the prognosis of T-ALL has gradually improved and cure rates have reached over 85% in children and ~50% in adults (2,3). However, the outcome of T-ALL patients with relapsed and refractory leukemia remains poor (1-3). Various novel therapeutic strategies have been recently studied, including tyrosine kinase inhibitors (TKIs) for the treatment of breakpoint cluster region-Abelson (BCR-ABL)-positive T-ALL and γ-secretase inhibitors (GSIs) for the treatment of T-ALLs with aberrant Notch 1 activation (4). Arsenic compounds have also been previously used to treat T-ALL cell lines and patients (5-8). Several studies have demonstrated that a safe and effective serum concentration of arsenic trioxide (ATO; 2-6 µmol/l) for treating acute promyelocytic leukemia (APL) patients could induce apoptosis in T-cell leukemia cell lines, notably in the Molt-4 cell line, or in leukemia cells from...
glucocorticoid-resistant ALL patients, independently or in combination with other agents, such as glucocorticoids (6-9). The combination of ATO and other common chemotherapy drugs provides a therapeutic target that may potentially be used to induce the remission of relapsed or refractory T-ALL patients.

During normal T-cell development, the earliest established T-cell lineage, immature cluster of differentiation (CD)34+ cells, enter the thymus and subsequently differentiate into mature T-cells, gaining a functional T-cell receptor (TCR) that belongs to the αβ or γδ lineage (10). T-cells implicated in T-ALL are characterized by the clonal expansion of malignant T-cells arrested at an early stage during T-cell differentiation (1). Clonally expanded malignant T-cells (leukemic clones) vary in certain patients due to TCR gene rearrangement diversity (11,12). The combination of reverse transcription (RT)-polymerase chain reaction (PCR) and the GeneScan technique, also referred to as ‘immunoscope’, has been widely used to analyze TCR repertoires and dynamically monitor clonal changes in T-cells in patients with leukemia and other diseases, including autoimmune diseases and certain types of viral infections (13,14). Using this method, the immune status of patients could be characterized and the evolution of malignant T-cell clones identified, which may aid in monitoring minimal residual disease (MRD) and designing specific therapeutic strategies for T-ALL (12,15). The present study reports a rare case of T-ALL, whereby the patient responded poorly to standard chemotherapy but achieved complete remission (CR) following treatment with protocols involving arsenic. In order to evaluate the effects of the treatment and monitor the reconstitution of the immune system following treatment with arsenic-combined regimens, the TCR, γ and δ repertoires of the T-ALL patient were monitored at 7 time points, between the time of diagnosis and when CR was achieved, over 4 months.

Case report

A 28-year-old male patient presented to Department of Hematology, Guangdong General Hospital (Guangzhou, China) due to dizziness in January 2013. The physical examination disclosed lymphadenopathy, splenomegaly and hepatomegaly, and no skin lesions were present. A complete blood count revealed a white blood cell count of 12x10⁹/l (normal range, 4-10x10⁹/l). Cytogenetic analysis showed a normal male karyotype 46XY with 5 aneuploids. In the peripheral blood (PB) and bone marrow (BM) aspirate smears, a high percentage of blasts (70 and 84%, respectively) were detected (normal, <0.01% in PB and <2% in BM). For immunophenotyping analysis (FCM) revealed that lymphoblasts accounted for 98.3% of the 30,000 BM cells counted, the majority of which were positive for CD71, CD7, CD2, CD5, CD10, CD34, cytoCD3 and terminal deoxynucleotidyl transferase, and a number of which were positive for CD33, CD13, CD56, human leukocyte antigen-antigen D related, CD9, CD38 and sCD34, while CD4 and CD8 were not expressed (Fig. 1A; Table I). Fluorescence in situ hybridization (FISH) analysis showed no evidence for BCR-ABL, mixed-lineage leukemia gene (MLL) or fms related tyrosine kinase 3 (FLT3) gene rearrangements or deletions. Other laboratory tests included a basic metabolic panel, liver test and coagulopathy panel, and renal function, which were all unremarkable. The present case was diagnosed with T-ALL based on cytomorphology, immunohistochemistry and cytogenetic and molecular analysis.

The patient was admitted in January 2013 and started on one course of vincristine, 2 mg, once a day (days 1, 8, 15 and 22); daunorubicin, 68 mg, once a day (days 1, 2, 3, 15 and 16); cyclophosphamide, 1,200 mg, once a day (days 1 and 15); L-asparaginase, 10,000 u (days 11, 14, 17, 20, 23 and 26); dexamethasone, 10 mg, once a day (days 1-4 and 5) and 5 mg, once a day (days 15-28) (VDLCP) regimen, followed by cyclophosphamide, 500 mg, every 12 h (days 1, 2 and 3); daunorubicin, 85 mg, once a day (day 4); vincristine, 2 mg, once a day (days 4 and 11); prednisone, 40 mg, once a day (days 1-4 and 11-14) (HyperCVAD-A) protocol, which is a more intensive chemotherapy regimen (16,17). However, the patient had poor response to the initial chemotherapy consisting of one course of VDLCP and one course of HyperCVAD-A (Fig. 1B) (13,14). One course of cyclophosphamide, cytarabine and topotecan (CAT) chemotherapy [cyclophosphamide, 400 mg, every 12 h (days 1-3); cytarabine, 1,500 mg, once a day (days 2-6); topotecan, 2 mg, once a day (days 2-6)] was then administered; however, the response assessments unfortunately indicated minor remission. Considering the refractory situation of the present patient, who failed to respond to the first and second line chemotherapeutics, ATO (which is widely used for treating APL and may be used as a saving treatment refractory leukemia cases) was then administered as salvage chemotherapy for the present patient. ATO (10 mg; 0.16 mg/kg/day) was administered by intravenous drip for 2-3 h once a day, and treatment was continued for 3 weeks. On August 29, 2013, the patient surprisingly achieved CR with MRD, with 2.8% of blast T-cells identified in the BM by FCM (Fig. 1C; Table I). The patient was continued on cyclophosphamide, 400 mg, every 12 h (days 1 and 2); topotecan, 2 mg, every day (days 2-4), oral arsenic (10 mg, once a day), methylprednisolone (MP; 100 mg, at night), and mitoxantrone and cytarabine (HyperCVAD-B), alternated with consolidation therapy, which enabled the patient to maintain CR without MRD (Fig. 1D; Table I). Arsenic-related hematological toxicity or extra-hematological toxicities were not observed during the time of treatment. The treatment details and information regarding the seven bone marrow samples collected at various time points are summarized in Table I.

To monitor the T-cells in the BM of the present patient during the burden of disease and evaluate the effects of treatment, repertoire-specific PCR primers and the GeneScan technique were used in combination, as previously described (18-20). The TCR Vβ (Fig. 2), Vγ and Vδ (Fig. 3) repertoires were dynamically characterized in BM samples collected at various times (Table I). The result showed only a subset of the TCR Vβ...
family members (9-16) were detectable in each of the samples collected at various time points (Fig. 2), which is in contrast with healthy individuals, who express nearly all of the TCR Vβ repertoire subfamily members (18,21). Although the expression of all of the TCR Vγ and Vδ family members was not found in each sample (Fig. 3), the expression pattern did not appear to be significantly different compared with the healthy individuals from a previous study (19), particularly when the patient achieved CR. Significantly, oligoclonally expanded T-cells were detected in certain TCR Vβ (Vβ1, Vβ21 and Vβ24) and Vδ (Vδ3 and Vδ6) subfamilies, while the majority of TCR subfamily T-cells displayed a polyclonal pattern. The evolution of T-cell clones was characterized at various time points prior to and following chemotherapy, particularly for the oligoclonally-expanded TCR subset, to detect a factor associated with outcome and to identify a malignant T-ALL clone.

Table I. Sample and clinical therapy details for the present patient with refractory T-cell acute lymphoblastic leukemia.

<table>
<thead>
<tr>
<th>Sample collection</th>
<th>Date of collection</th>
<th>Date of therapy</th>
<th>Type of therapy</th>
<th>Smear analysis blast cells in BM/PB, %</th>
<th>FCM analysis blast cells in BM, %</th>
<th>Disease status at sample acquisition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>31.01.2013</td>
<td></td>
<td></td>
<td>84/70</td>
<td>98.3</td>
<td>ND</td>
</tr>
<tr>
<td>B</td>
<td>20.02.2013</td>
<td>04.02 - 28.02.2013</td>
<td>VDLCP</td>
<td>79/66</td>
<td>43.3</td>
<td>MR</td>
</tr>
<tr>
<td>C</td>
<td>03.04.2013</td>
<td>04.04 - 17.04.2013</td>
<td>HyperCVAD-A</td>
<td>62/5</td>
<td>73.3</td>
<td>MR</td>
</tr>
<tr>
<td>D</td>
<td>27.05.2013</td>
<td>29.05 - 22.06.2013</td>
<td>CAT</td>
<td>92/40</td>
<td>MR</td>
<td>MR</td>
</tr>
<tr>
<td>E</td>
<td>29.08.2013</td>
<td>29.08 - 26.10.2013</td>
<td>ATO</td>
<td>4/0</td>
<td>2.8</td>
<td>CR+MRD</td>
</tr>
<tr>
<td>F</td>
<td>31.10.2013</td>
<td>31.10 - 05.11.2013</td>
<td>ATO+CT</td>
<td>2/0</td>
<td>0.0</td>
<td>CR</td>
</tr>
<tr>
<td>G</td>
<td>30.12.2013</td>
<td>03.01 - 24.01.2014</td>
<td>ATO+MP</td>
<td>2/0</td>
<td>0.0</td>
<td>CR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>03.28 - 01.04.2014</td>
<td>ATO+CT</td>
<td>2/0</td>
<td>0.0</td>
<td>CR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.07 - 18.07.2014</td>
<td>ATO+HyperCVAD-B</td>
<td>1/0</td>
<td>0.0</td>
<td>CR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.07.2014 - present</td>
<td>ATO</td>
<td></td>
<td></td>
<td>CR</td>
</tr>
</tbody>
</table>

FCM, flow cytometry; BM, bone marrow; PB, peripheral blood; VDLCP, vincristine, daunorubicin, L-asparaginase, cyclophosphamide and dexamethasone; HyperCVAD-A, cyclophosphamide, vincristine, doxorubicin and dexamethasone; CAT, cyclophosphamide, cytarabine and topotecan; ATO, arsenic trioxide; CT, cyclophosphamide and topotecan; MP, methylprednisolone; HyperCVAD-B, mitoxantrone and cytarabine; ND, newly diagnosed; MR, minor remission; CR, complete remission; MRD, minimal residual disease.

Figure 1. FCM for CD7 and HLA-DR expression analysis of the bone marrow at various disease states. Red scatter points represent the lymphoblast in bone marrow. Pink, green, blue, yellow and gray dots represent nucleated erythrocytes, mature lymphocytes, monocytes, pre-B lymphocytes and mature granulocytes, respectively, which gated in 2D spot figure (CD45-Perp and Side Scatter-Height). (A) Time of new diagnosis with T-cell acute lymphoblastic leukemia. FCM detected 98.3% blast cells, and the majority of the blast cells were positive for CD7 and HLA-DR. (B) Time of achieving minor remission following treatment with the vincristine, daunorubicin, L-asparaginase, cyclophosphamide and prednisone regimen and the cyclophosphamide, vincristine, doxorubicin and dexamethasone regimen. FCM detected 73.3% blast cells. (C) Time of achieving CR following treatment with ATO. FCM detected 2.8% MRD and few cells were positive for CD7 and HLA-DR. (D) Time of CR that was maintained for 4 months in which no significant MRD could be detected by FCM. Scarce cells positive for CD7 and HLA-DR were found. FCM, flow cytometry; CD7, cluster of differentiation 7; HLA-DR, human leukocyte antigen-antigen D related; CR, complete remission; MRD, minimal residual disease; FITC, fluorescein isothiocyanate.
clone product was 184 bp in size, while the 190 bp product remained present as a minor clone (Fig. 4F). Notably, the Vβ1 cell clone product of 190 bp became the major clone again 4 months subsequent to CR (Fig. 4G). At the time, MRD was not detected by FCM; however, the relative fluorescence intensity of Vβ1 was remarkably decreased compared with samples collected in the stage without CR (data not shown). In addition to the Vβ1 subfamily, oligoclonality or an oligoclonal trend could also be detected for the Vβ21 or Vβ24 subfamily, which had various product sizes or clones for certain patient samples. Overall, based on the evolution of the Vβ1 T-cell clones, the Vβ1 T-cell clone appearing with the 190-bp product may be the malignant T-ALL clone, as the numbers of this clone decreased following chemotherapy, particularly ATO therapy.

Malignant T-ALL clones may express alternative TCRαβ or γδ receptors (8); therefore, the TCR Vγ and Vδ repertoires were also analyzed in all samples of the present case. Since skewed Vγ and Vδ subfamily distribution is a common characteristic of the leukemia patients (19), similar results were expected and detected in the present study. In the present case, clonally expanded Vδ3 T-cell clones of the same size as CDR3 were identified in all samples at various time points (Fig. 3); however, no Vγ subfamily members were detected in 3 of the samples (Fig. 3). This inconsistency may be due to a relatively low frequency of γδ+ T-cells in the samples, which were not detected using the present techniques. Furthermore, it appears unlikely that the Vδ3 T-cell clone is the malignant T-ALL clone in this case, as Vγ subfamily members that should pair with Vδ to form γδ+ T-cell clones could not be detected at the time of diagnosis (Fig. 3A), which also supports the theory that malignant T-ALL clones should be αβ+ T-cell clones expressing Vβ1.

Written informed consent was obtained from the patient for publication of the present study and any accompanying images.

Discussion

The present study may be the first documented case of a patient with T-ALL that achieved CR by ATO induction treatment, although the achieved CR may not hold true on a molecular level (indicated by the results of the T-cell repertoire analysis). Medicinal uses of arsenic have been documented for >2,000 years. ATO has been previously shown to be dramatically effective for treating patients with APL (22,23), and is also effective in ~20% of patients with myelodysplastic syndrome (MDS) (24,25). The mechanisms of ATO-induced apoptosis include the degradation of promyelocytic leukemia (PML)-retinoic acid receptor α (RARα), which may occur with or without the interference of B cell lymphoma-2 (Bcl-2) family genes (8,26,27). Several other mechanisms of ATO anti-leukemic effects have been identified, including the
induction of PML-RARα-independent apoptosis, cell cycle arrest, growth inhibition, induction of stress related processes, direct mitochondrial damage and the inhibition of nuclear factor-κB (NF-κB) (8,26-31). Based on the dramatic effects of ATO on APL and the partial effects on MDS, several studies have tested the efficacy of ATO on other malignant T-cell lines. ATO has been demonstrated to selectively inhibit growth and induce apoptosis in several cell lines, including the megakaryocytic leukemia cell lines HEL, Meg-01, UT7, and M07e (32), the myeloid leukemia cell lines U937 and KG-1, plasma cells and cell lines from myeloma patients (33) and B cell leukemia cell lines (34). Notably, ATO has been demonstrated to be capable of inducing apoptosis in certain solid tumor cells, for example non-small cell lung cancer cells and sarcoma cells (35,36), demonstrating the broad antitumor activity of arsenic. Due to a lack of effective treatments against adult T-cell leukemia/lymphoma (ATLL), ATO activity was also tested in vitro in several HTLV-1-infected cell lines. In 1998, a Japanese group conducted the first in vitro studies on the comparative effects of ATO and retinoic acid (RA) on HTLV-1-infected cell lines and fresh ATL cells (37). Subsequently, several in vitro studies indicated that ATO combined with interferon-α (IFN-α) is a promising therapy for ATLL (38-41). The surprising specificity of the cellular effects of ATO, by exclusively targeting the viral oncoprotein p40, human T-lymphotropic virus (Tax) and the NF-κB pathway, provided a biological basis for dual IFN-α/As₂O₃ treatment for ATLL patients (42). At present, several studies have reported phase II trial results of dual IFN-α/As₂O₃ treatment in relapsed/refractory ATLL (23,43,44). The majority of these studies exhibited feasible anti-leukemia effects in ATL patients with a poor prognosis, even though the treatment context should be optimized (23,43,44). In addition, arsenic, IFN-γ and zidovudine were shown to be important.
With the promising role of arsenic and arsenic combined with dual or triple treatment in ATLL, and the specific effects of arsenic compounds in the treatment of APL, one study investigated the potential of using arsenic to treat relapsed or refractory ALL (5). The disappointing result showed that ATO was not active in the treatment of ALL in a cohort of 11 patients [5 early pre-acute B-cell lymphoblastic leukemia (B-ALL), 2 pre-B-ALL, 1 mature B-ALL and 3 T-ALL] in 2006 (5). However, this result should not preclude additional evaluation of arsenic in combination therapies for ALL. For example, a previous study reported that subtoxic doses of ATO and glucocorticoids could be advantageous for the treatment of glucocorticoid-resistant ALL cells from T- and precursor B-ALL patients (6). The underlying pro-apoptosis mechanism partially depended on the inhibition of the protein kinase B/X-linked inhibitor of apoptosis protein pathway and the activation of the pro-apoptosis Bcl-2 family member, Bcl-2 associated agonist of cell death (6). In addition, the cytotoxicity effect with an 80% inhibition rate of ATO in the Molt-4 cell line was reported to involve apoptosis and autophagy via the upregulation of Beclin-1 at the post-transcriptional level (8,9). The pre-clinical evidence indicated that ATO alone or combined with other chemotherapy regimens have a potential capability in treating T-ALL patients via complicated mechanisms (6,8,9). In the present study, the T-ALL patient had a poor response to induction remission regimens, so the patient was treated with a protocol involving arsenic, which is a salvaging treatment for refractory leukemia patients in the Department of Hematology, Guangdong General Hospital. Notably, the patient responded well to arsenic treatment regimens without evident toxicity. At present, it is unknown whether the successful treatment of the present case was due to the therapeutic strategy (drug-dose, route of administration or drug-combination) or to factors that rendered the patient sensitive to arsenic treatment. However, the present rare case at least provides support for the use of arsenic agents combined with other drugs, such as glucocorticoids, to treat T-ALL, as alternative salvage chemotherapy in the future.

In the present case, the restricted expression of the TCR Vβ, Vγ and Vδ repertoire subsets displayed a common characteristic of samples collected at various time points and various disease states. For example, restricted use of the TCR Vβ subfamilies lacking Vβ2, 5, 7, 10, 17 and 22 was shown (Fig. 4B). Previous studies have shown that TCR repertoire deficiency is a common characteristic of patients with leukemia, including those with T-ALL (12,46). Potential reasons for deficiencies in the TCR repertoire include: i) The prior proliferation of a malignant T-cell clones suppressed the proliferation of normal T-cell clones, and ii) the tumor microenvironment or other unknown factors affect the competency of the immune system (47,48). However, the T-cell repertoire deficiency was not significantly reconstituted even when the patient achieved CR, as the reconstitution of the TCR repertoire is slow due to factors that rendered the patient sensitive to arsenic treatment. However, the present rare case at least provides support for the use of arsenic agents combined with other drugs, such as glucocorticoids, to treat T-ALL, as alternative salvage chemotherapy in the future.

**Figure 4. Dynamic changes in complementarity determining region 3 spectratyping of Vβ T-cells in samples at various time points. The horizontal axis represent the product size and the height of a single peak in each graph represents the fluorescence intensity of product in corresponding size. (A) Time of new diagnosis with T-cell acute lymphoblastic leukemia. (B) Time of achieving MR following treatment with the vincristine, daunorubicin, L-asparaginase, cyclophosphamide and prednisone regimen. (C) Time of achieving MR prior to treatment with the HyperCVAD-A regimen. (D) Time of achieving MR following treating with the HyperCVAD-A regimen. (E) Time of achieving CR but with MRD upon treatment with cyclophosphamide, cytarabine and topotecan and ATO. (F) Time of achieving CR with no MRD detected following treating with a second course of ATO. (G) Time of CR that was maintained for 4 months in which no significant MRD could be detected by flow cytometry. ND, newly diagnosed; MD, minor remission; CR, complete remission; MRD, minimal residual disease; HyperCVAD-A, cyclophosphamide, vincristine, doxorubicin and dexamethasone; ATO, ATO, arsenic trioxide.**
Unlike T-ALL patients following HSCT, in which T-ALL clones are eradicated and not detected at the molecular level by GeneScan analysis, Vβ T-cell clones of the same size could be detected at all time points in the present case, even when the patient achieved CR lasts for >4 months. This result suggests that molecular CR cannot be achieved in cases treated with arsenic and chemotherapy. The result also has significant implications for MRD monitoring using specific clonal T-cell detection by GeneScan and RT-PCR, which may provide dynamic information for disease states and direct further therapy (12).

In the majority of cases with cancer or leukemia, an antitumor T-cell clone could be identified in patient samples (blood, BM or tumor tissue) even if the patient had immunodeficiency (46,50). In the present study, clonally expanded Vβ21, Vβ24, Vδ3 and Vδ6 were identified in certain samples collected from various time points, and whether such clonally expanded T-cells are special responders to T-ALL therapy requires further investigation.

In conclusion, the present reported that arsenic induced CR in a case with refractory T-ALL, which supports an alternative salvage therapeutic method for clinically treating refractory and relapsed T-ALL patients. However, the prolonged response to arsenic associated with treatment and the associated toxicity requires additional studies. The distribution and clonality of the TCR β, γ and δ repertoires were characterized in samples from various time points from the patient. The results suggested that the evolution of a malignant T-ALL clone occurred, indicating that arsenic therapy may be unable to induce molecular CR in T-ALL patients. Therefore, dynamically monitoring the TCR repertoire distribution and clonal evolution combined with clinical course analysis would aid in predicting the prognosis of the patient and in designing specific therapeutic strategies.

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