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Diffuse large B-cell lymphoma successfully treated with amplified natural killer therapy alone: A case report

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Abstract

BACKGROUND

The prognosis of patients with advanced diffuse large B-cell lymphoma (DLBCL) is poor, with a 5-year survival rate of approximately 50%. The mainstay of treatment is multidrug combination chemotherapy, which has been associated with serious side effects. Amplified natural killer (ANK) cell therapy amplifies and activates natural killer (NK) cells to attack only malignant tumors. As ANK cells attack programmed death ligand 1 (PD-L1)-positive tumor cells, ANK therapy is considered effective against adult T-cell lymphoma (ATL) and malignant lymphoma.

CASE SUMMARY

Herein, we report a case of an older patient with advanced DLBCL who was successfully treated with ANK immunotherapy. A 91-year-old female visited our hospital with sudden swelling of the right axillary lymph node in April 2022. The patient was diagnosed with stage II disease, given the absence of splenic involvement or contralateral lymphadenopathy. ANK therapy was administered. Six rounds of lymphocyte sampling were performed on July 28, 2022. To reduce the occurrence of side effects, the six samples were diluted by half to obtain 12 samples. Cultured NK cells were administered twice weekly. The treatment efficacy was evaluated by performing computed tomography and serological tests every 1 or 2 mo. The treatment

suppressed lesion growth, and the antitumor effect persisted for several months. The patient experienced mild side effects. PD-L1 immunostaining was positive, indicating that the treatment was highly effective.

CONCLUSION

ANK therapy can be used as a first-line treatment for malignant lymphoma; the PD-L1 positivity rate can predict treatment efficacy.

INTRODUCTION

Malignant lymphomas are hematopoietic tumors that originate from mature lymphocytes. Historically, Hodgkin's first described Hodgkin's lymphoma in 1834. Malignant lymphomas are broadly classified into those derived from B lymphocytes (B-cell) and those derived from T lymphocytes or natural killer (NK) cells (T/NK cells). Currently, the World Health Organization classification is widely used for categorizing this disease[1]. Malignant lymphomas are further classified into three types based on the general growth rate before treatment: slowly progressive (indolent), rapidly progressive, and very rapidly progressive. In Japan, malignant lymphoma impacts approximately 35,000 individuals annually, with an incidence rate of 28 cases per 100,000 individuals and 13,000 deaths estimated annually[2].

The present case report discusses diffuse large B-cell lymphoma (DLBCL), the largest category of non-Hodgkin's lymphoma. Morphologically, DLBCL is characterized by diffuse proliferation of medium- to large-sized cells, and a heterogeneous case population may develop DLBCL due to histological transformation during the course of follicular or mucosa-assisted lymphoid tissue lymphoma[3]. Malignant lymphoma is diagnosed by histopathological examination of biopsy samples of the lesion. If malignant lymphoma is suspected, surgical incisional biopsy is preferred to obtain a pathological specimen of sufficient size. In addition, ancillary tests,

such as flow cytometry, chromosome testing (including the fluorescence in situ hybridization method), and various genetic tests are performed when feasible.

DLBCL is classified into germinal center B-cell-like and activated B-cell-like subtypes based on differences in gene expression derived from cancer cells[4,5]. Furthermore, disease classification can be achieved using the Ann Arbor classification[6]. The International Prognostic Index of the National Comprehensive Cancer Network has been proposed as a prognostic model[7]. Treatment type is determined based on a combination of these factors. Drug therapy forms the treatment basis, which depends on the type of disease. The main pharmacotherapeutic approach involves combination chemotherapy comprising cytotoxic antineoplastic agents: rituximab, cyclophosphamide, vincristine, and prednisolone (R-CHOP) or bendamustine and rituximab (BR) therapy for B-cell lymphoma, and doxorubicin, bleomycin, vinblastine, and dacarbazine (ABVD) therapy for Hodgkin's lymphoma. Radiotherapy may also be administered depending on the location of the lesion.

The recommended treatment for early and advanced-stage DLBCL is 6–8 courses of R-CHOP therapy[8,9]. Regarding prognosis, the 5-year survival rate for early and advanced DLBCL is 58%. However, the prognosis remains markedly poor in the case of resistance to initial treatment or early recurrence. CD19-targeted chimeric antigen receptor (CAR)-T-cell therapy has recently been developed. However, the 1-year survival rate is limited to ~50%, and the therapeutic effect is insufficient, accompanied by serious side effects such as cytokine release syndrome, encephalopathy, neurotoxicity, and cytopenia. Hence, a preferred treatment strategy is currently lacking [10].

In 1985, Rosenberg *et al* introduced immunotherapy at the U.S. National Cancer Institute, developing a treatment called lymphokine-activated killer (LAK) cell immunotherapy[11]. In LAK cell immunotherapy, a large volume of blood, approximately 50 L, is drawn from the patient over five days in a single week, and the extracted lymphocytes are cultured with recombinant interleukin (IL)-2 for 3–4 days to induce LAK cells, which are subsequently injected into the patient over a short period.

Although this treatment showed a certain level of efficacy, it is not commonly available owing to its high cost and intense side effects. The amplified NK (ANK) immunotherapy used in the current case focuses on the fact that NK cells exert the strongest anti-cancer activity among various lymphocytes. The amount of blood collected is ~5 L; however, by increasing the number and activity of NK cells and returning them to the patient, a safe and highly effective treatment can be provided.

Theoretically, ANK immunotherapy is effective against all cancers. This unique treatment method, researched and developed by Lymphocyte Bank Co., Ltd., is distinct from conventional immunotherapy, including LAK and CAR-T-cell therapy[12,13,14].

CASE PRESENTATION

Chief complaints

A 91-year-old female visited our hospital with sudden swelling of the right axillary lymph node.

History of present illness

The symptom was observed in April 2022.

History of past illness

The patient developed reflux esophagitis and a hiatus hernia at 85 and 86 years of age, respectively

Personal and family history

No relevant family history was reported.

Physical examination

No date

Laboratory examinations

Detailed laboratory data is presented in Table 1. Puncture cytology and culture of the right axillary lymph node showed no malignant findings nor any findings suspicious of mycobacterium infection. Given that the hematologic examination revealed the presence of toxoplasma IgG antibodies and the patient had a cat, the condition was initially judged to be lymphadenopathy due to acute toxoplasmosis, and antimicrobial treatment was administered. The tumor temporarily shrank, but the right axillary lymph node appeared swollen again after approximately a month, accompanied by enlargement of the subclavian lymph node (Figure 1). Hence, a right subclavian lymph node biopsy was performed. Histological examination of the sample showed pathological results of follicular lymphoma or DLBCL (Figure 2). For differentiation, immunostaining was performed, and CD20(+), CD79a(+), CD10(+), bcl2, and bcl6(+) were detected (Figure 3).

Imaging examinations

Figure 1 Imaging findings at diagnosis. A: Subclavian lymph node enlargement; B: Right axillary lymph node enlargement.

Figure 2 Pathological findings at diagnosis. A: HE ×10; B: HE ×40.

HE, hematoxylin-eosin.

Figure 3 Immunostaining at diagnosis. A: CD20 ×40(+); B: CD79a ×40(+); C: CD10 ×40(+); D: bcl6 ×20(+); E: bcl2 ×20(+).

Figure 4 Course of LDH, IL-2, and ANK treatment schedule.

ANK, amplified natural killer; IL-2, interleukin-2; LDH, lactate dehydrogenase

Figure 5 Post-treatment course of CT imaging. Three months after treatment, the size of the right axillary and subclavian lymph nodes appears to be gradually reduced; five months later, a marked reduction can be observed. A: 2022/7; B: 2022/10; C: 2022/12; D:2023/2; E: 2023/3. CT, computed tomography

FINAL DIAGNOSIS

The patient was diagnosed with stage II disease owing to a lack of splenic involvement or contralateral lymphadenopathy. Disease severity was low-to-moderate according to the International Prognostic Index (IPI), considering age and serum LDH levels.

TREATMENT

Given that the patient was over 90 years of age, adapting the standard of care for DLBCL (drug combination chemotherapy) to her advanced age posed considerable challenges. Based on the preference for limited side effects and increased therapeutic effects, as requested by the patient's family, we decided to initiate ANK therapy. The patient was highly motivated to receive treatment, and despite the rapidly growing right axillary lymph node, she engaged well with activities of daily living.

Six rounds of lymphocyte sampling were performed on July 28, 2022. Considering the patient's advanced age, the six samples were diluted to half the initial concentration to achieve 12 samples to reduce the occurrence of side effects. The cultured NK cells were administered twice weekly (Figure 4) *via* outpatient care. The treatment efficacy was evaluated by performing computed tomography (CT) and serological tests every 1 or 2 mo.

OUTCOME AND FOLLOW-UP

At one month, the right axillary lymph node was mildly enlarged, and the subclavian lymph node size was unchanged (Figure 5b). IL-2 and LDH levels were 2,614 U/mL and 261 U/L, respectively (Figure 4). At two months, the size of the right axillary lymph node showed a prominent reduction, and the subclavian lymph node showed a mild reduction (Figure 5c). IL-2 and LDH levels were 2,174 U/mL and 255 U/L, respectively (Figure 4). At five months, the right axillary lymph node, which initially was the size of a fist, had shrunk to the size of the head of a thumb. The subclavian lymph node also shrank remarkably; the swelling was substantially reduced, such that the site of swelling was imperceptible (Figure 5d). At six months, the size of the axillary and subclavian lymph nodes showed further reduction (Figure 5e), and the IL-2 and LDH

levels were 233 U/mL and 185 U/L, respectively (Figure 4). Although it is necessary to carefully monitor the treatment progress to establish the duration of efficacy, we found that the treatment suppressed lesion growth from administration onward, and the antitumor effect persisted for several months. After administration, the patient experienced general malaise and fever, although the temperature did not exceed 37°C. The side effects were mild.

DISCUSSION

To the best of our knowledge, this is the first report of a markedly old patient diagnosed with DLBCL who was successfully treated with ANK therapy alone. Typically, the main drug therapy for DLBCL is combination chemotherapy comprising cytotoxic antineoplastic agents. It is well-established that chemotherapy is not indicated for markedly older patients owing to difficulties such as the occurrence of side effects, with most older patients receiving only palliative care. The established guidelines recommend repeat R-CHOP therapy in patients diagnosed with DLBCL and advanced-stage II Ann Arbor disease with a positive bulky mass at initial treatment[9,15]. Typical side effects of R-CHOP therapy include anorexia, nausea, constipation, numbness in the limbs, fever, hair loss, bone marrow suppression, and decreased renal function, known to pose a substantial burden on patients. Although the patient was in her 90s and markedly old, her cognition was sufficiently robust to allow decision-making regarding the treatment risks. According to the guidelines, combination chemotherapy has benefits and risks. A meta-analysis published in 2021 has reported the efficacy of R-CHOP. However, R-CHOP therapy involves multidrug chemotherapy with the potential to induce severe side effects; hence, it is considered a high-risk treatment, especially among older patients. Our team is well-versed in the efficacy and risks of ANK therapy. Patients and their families have previously requested ANK therapy, which is associated with fewer side effects than chemotherapy, can be received on an outpatient basis, and is expected to gain momentum as an effective treatment strategy[16-18].

It has been reported that NK cells extracted from blood and then cultured and activated can attack tumors better than T cells, regardless of tumor suppressor molecule expression. This finding implies that ANK therapy is tumor cell-specific and carries a low risk of serious damage to the normal immune system. ANK cells have been reported to kill PD-L1-positive tumor cells[19]. Kataoka *et al* have reported that structures with missing or elevated PD-L13'-UTR are frequently observed in ATL, inducing substantially high PD-L1 expression. Therefore, the therapeutic efficacy of ANK therapy is reported to be high. Considering side effects, serious side effects observed with the commonly used anti-CCR4 antibody have not been reported with ANK therapy[20-22]. Therefore, it differs from existing immunotherapies in terms of efficacy and safety.

In addition to ATL, other cancers have shown similar genomic abnormalities, with such anomalies frequently observed in diffuse large-cell lymphoma, gastric cancer, esophageal cancer, and cervical cancer[21]. These findings suggest that repeated administration of NK cells, including ANK cells, can alleviate immunosuppression *via* the PD-1-PD-L1 pathway[22,23]. Regarding DLBCL, immunostaining studies of intravascular large B-cell lymphoma have reported a high rate of PD-L1 expression[24].

Based on the abovementioned prior reports, we considered that ANK therapy would be more effective in tumor cells with high PD-L1 positivity. Pathological tissues derived from the patient were subjected to PD-L1 immunostaining (Figure 6), revealing positive immunostaining.

Accordingly, in the current patient, DLBCL was associated with a high number of PD-L1-positive tumor cells, and ANK therapy was likely to be very effective. Notably, the results were markedly good, and ANK therapy was administered twice weekly for a total of 12 times from around August, when the treatment was initiated, to the end of September. Accordingly, the lesion growth was suppressed following the administration of ANK therapy. Approximately three months later (around December), the right axillary and right subclavian lymph nodes began to shrink. After approximately five months of treatment, all lymph nodes had shrunk markedly. IL-2

Levels had also decreased from 2,000 to 200 U/mL, nearing remission. Six months later, the tendency of lesion reduction was persistently maintained. Although the duration of efficacy of ANK therapy on DLBCL and the possibility of complete remission is yet to be confirmed, ANK therapy was markedly effective in treating DLBCL in the present case, more effective than multidrug chemotherapy combining 4–5 drugs, the typical therapeutic approach recommended by current guidelines. Importantly, ANK therapy induced less severe side effects than those induced by conventional chemotherapy, as evidenced in our patient.

CONCLUSION

ANK therapy could be effective in ATL and several other solid tumors. Considering the potential mechanism, it can be postulated that the greater the number of PD-L1-positive tumor cells, the more effective the therapy. These points have been successfully demonstrated in the current case report[9,24,20]. Although additional cases need to be accumulated to determine and confirm the efficacy and side effects of ANK therapy, it could be used as a first-line therapy to replace R-CHOP therapy in treating malignant lymphoma. Moreover, PD-L1 could be an effective biomarker to establish the efficacy of ANK therapy in other types of cancers.

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