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Case Report

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# Efficacy and Future Prospects of ANK Therapy for ATL, Malignant Lymphoma and Solid Tumors

**Kenjiro Nagai[1,2*], Syo Nagai[1], Yuji Okubo[3] and Keisuke Teshigawara[3]**

[1]*Medical Corporation Ebino Centro Clinic, Ebino, Japan*

[2]*Department of Respiratory Medicine, Yokohama City University, Yokohama, Japan*

[3]*Higashinotoin Clinic, Kyoto, Japan*

## Abstract

Amplified Natural Killer Cell (ANK) therapy has been modified to enhance the safety and efficacy of original (LAK) immunotherapy. This is a method of removing Natural Killer (NK) cells from the patient's own blood, culturing and amplifying the NK cells, increasing their ability to specifically attack cancer, and returning them for treatment. It is generally effective against all cancers. Experienced a case in which ANK therapy was remarkably effective against ATL, prostate cancer, and breast cancer. Treatment of ATL is basically chemotherapy, but it is not effective and has many side effects. Chemotherapy is also the main treatment for solid cancer patients whose condition has progressed in the same way, and the elderly, renal failure, and heart failure patients cannot be treated. ANK therapy is highly effective in ATL cases and is also very effective in certain solid tumor cases. Considering the mechanism of action of ANK therapy from the accumulation of cases so far and research reports so far, it is effective for ATL with many PD positive tumor cells because it effectively kills PD-L1 positive tumor cells. Some types of solid tumors, such as lymphoma, gastric cancer, lung cancer, breast cancer, and prostate cancer, have many PD-L1-positive tumor cells. By measuring PD-L1-positive tumor cells and treating those with high levels, it may be possible to provide treatments that are more effective, have fewer side effects, and are safer than existing treatments.

**Keywords:** Amplified Natural Killer cell therapy • Lymphokine-activated killer cell immunotherapy • Adult T-cell leukemia • PD-L1-positive tumor cells • Cancer

## Introduction

Amplified Natural Killer Cell (ANK) therapy is a method of removing Natural Killer (NK) cells from the patient's own blood, culturing and amplifying NK cells to specifically enhance their ability to attack cancer, and returning them to the patient for treatment. It is generally effective against all cancers. In 1985, Rosenberg et al of the National Cancer Institute (NCI) performed a treatment called Lymphokine-Activated Killer (LAK) immunotherapy [1]. A large volume of blood of about 50 L was extracted from the patient over 5 days in a week, and lymphocytes were extracted and cultured with rIL-2 for 3 to 4 days to induce LAK cells. These cells were then transfused back to the patient. This treatment has shown some effects, but it is not common due to its high cost and strong side effects. The next instance where this therapy was performed was in Japan in the 1990s. However, the amount of blood collected was less than one-tenth of that collected by Rosenberg et al. Moreover, the expected effect was not achieved because the number of NK cells with a strong anticancer activity was low [2]. ANK immunotherapy focuses on the fact that among the various lymphocytes, NK cells have a strong anticancer effect. The amount of blood collected in the studies was about 5 L; however, by increasing the number and activity of NK cells before returning them to the patient, it is possible to obtain a safe and therapeutically useful effect [3]. ATL is caused by Human T-cell Leukemia Virus type 1 (HTLV-1). This cancer is a poor prognosis peripheral T-cell tumor that arises in HTLV-1 carriers. It was first reported by Uchiyama, Takatsuki et al. In 1977, the RNA retrovirus HTLV-1 was identified as the causative virus in his T-cell tumor outbreak in 1980 in southwestern Japan [4,5]. Based on various pathological factors and the clinical course of the disease, it is divided into 4 types: acute type, lymphoma type, chronic type, and smoldering type. Acute/lymphoma type and chronic type ATL with poor prognostic factors are classified as aggressive ATL, and chronic type/smoldering type ATL without poor prognostic factors are classified as indolent ATL. Treatment methods are decided on the basis of these classifications [6].

Aggressive ATL has a very poor prognosis with a median survival of approximately 10 months. Multidrug chemotherapy, and if possible, allogeneic haematopoietic stem cell transplantation may be administered, considering age and general condition. Smouldering ATL is treated with topical treatments such as corticosteroids, external retinoids, local radiation therapy, and photo chemotherapy of skin lesions. Systemic treatments include systemic administration of steroid hormones, oral retinoids, interferon-γ, and single-agent chemotherapy. However, they have little effect on prolonging survival and have strong side-effects. Amplified Natural Killer Cell (ANK) immunotherapy is different from conventional immunotherapy in that it focuses on the strong anti-cancer effect of Natural Killer (NK) cells from various lymphocytes. In this method, NK cells are taken from the patient's blood, cultured to specifically enhance the cancer-fighting function, and returned to the body to start treatment. This method is generally considered to be effective against all cancers.

This time, we will show cases of ATL, prostate cancer, and breast cancer that showed remarkable effects, and evaluate the efficacy and safety of ANK therapy. In addition, we consider which cases are more effective and suitable for treatment.

## Case Presentation

### Cases of ATL

**Case 1:** It was a 70-year-old woman who we believe switched from

***Corresponding author:** Dr. Kenjiro Nagai, Medical Corporation Ebino Centro Clinic, Ebino, Japan; Tel: 0984-33-5777; E-mail: ken701n427@gmail.com*



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smoldering-type to acute-type ATL. Originally a patient with ringworm infection, the patient was diagnosed with smoldering ATL in 2004. In 2007, the patient had a sharp rise in soluble IL-2 receptor levels (sIL-2R) in the serum (Figure 1), an increase in systemic lymph node sizes (Figure 2A), and exacerbation of the disease due to skin tumors (Figure 2B).

The patient's blood contained abnormal cells of lymphocytes (27%). We determined that it was a conversion from the smoldering type to the acute type. Then, the patient was treated with ANK therapy with over 15 injections from November 12, 2007 to February 15, 2008 (Table 1). This case also showed a dramatic downregulation of sIL-2R levels (Figure 2A). The patient's skin tumor also disappeared (Figure 2C), inducing complete remission (Table 1) (Figures 1 and 2A-2C) [3].

**Case 2:** It was an 81-year-old woman diagnosed with HTLV-1-associated bifurcated bronchoalveolar disease (HABA-B) with smoldering ATL.

She also tested positive for HTLV-1 antibodies and by line blotting (a test similar to Western blotting). Serological tests revealed <5% atypical lymphocytes, a lymphocyte count <4000/μL, and a Lactate Dehydrogenase (LDH) level <1.5 times normal. Serum calcium levels were normal (Table 1). Bronchoscopy revealed lymphocytic infiltration from bronchial mucosal tissue, consistent with her HTLV-1-associated bronchoalveolar injury (Figure 3).

Based on the above, the patient was diagnosed with smoldering ATL+HABA-B. Because the patient had severe respiratory distress and was elderly, ANK therapy (Figure 4) was administered. Symptoms improved dramatically and the patient's condition improved as seen on chest CT images and pulmonary function test results (Table 2) (Figures 4 and 5) [7] .

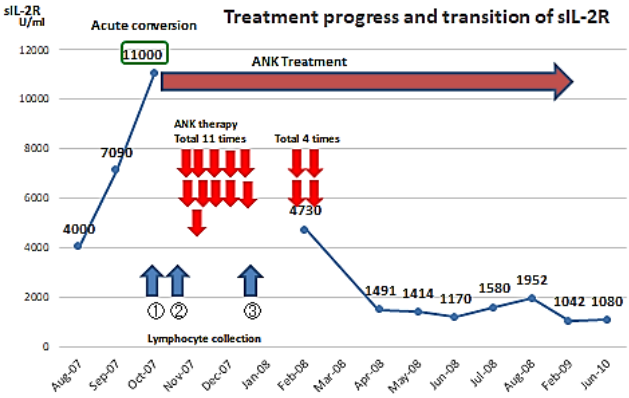


**Figure 1.** Serum soluble IL-2 receptor (sIL-2R) in patients and treatment course of ANK therapy. **Note:** (—) sIL-2R; (⬇) ANK therapy; (⬆) Lymphocyte Collection.

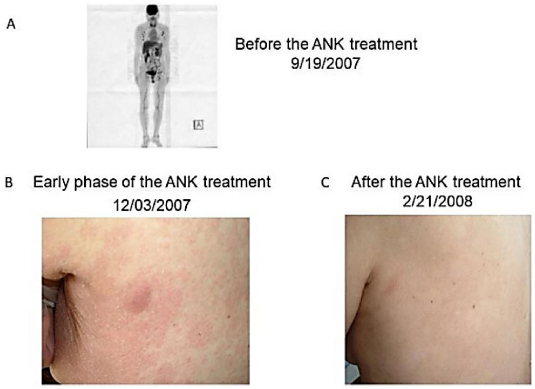


**Figure 2.** Positron Emission Tomography-Computed Tomography (PET-CT) images (before ANK cell treatment) and patient skin lesions. A. PET-CT showed multiple lymphadenopathy before ANK cell treatment; B. During the initial stage of ANK cell therapy, rashes and tumors were observed all over the body; C. Multiple skin lesions healed after repeated administration of ANK cells.

**Table 1.** Laboratory data at initial visit.

| Hematology | | Serology | |
|---|---|---|---|
| WBC | 9540/μL | ACE | 13.8/μL |
| | | RF | 4 IU/mL |
| Neutrophils | 59% | KL-6 | 177 U/mL |
| Lymphocytes | 37% | sIL-2 | 391 U/mL |
| Mono | 4% | IgG | 1845 mg/dL |
| Eos | 0% | IgA | 351 mg/dL |
| ATL | 5% | IgE | 2 IU/mL |
| Flower cells | 0% | HTLV-1 Ab | (+) |
| RBC | 3.64 × 106/μL | HTLV-1 LIA | (+) |
| Hb | 10.6 g/dL | P.19 Ab | (+) |
| Plt | 195 × 10/μL | P.24 Ab | (+) |

| | | | |
|---|---|---|---|
| Biochemistry | | P.46 Ab | (+) |
| TP | 7.3 g/dL | P.21 Ab | (+) |
| Alb | 4.1 g/dL | T-SPOT | (-) |
| BUN | 16.6 mg/dL | β-D glucan | 2.8 pg/mL |
| Cre | 0.62 g/dL | Cold agglu | (-) |
| Ca | 9.3 g/dL | Intratracheal sputum bacteria culture | |
| IP | 3 g/dL | MSSA (S. aureus) | |
| T-Bil | 0.7 mg/dL | Intratracheal sputum acid-fast bacilli culture | |
| AST | 26 U/L | (-) | |
| ALT | 11 U/L | Non-tuberculous mycobacteriosis PCR test | |
| ALP | 156 U/L | Mycobacterium avium (-) | |
| γGTP | 11 U/L | Mycobacterium intraceller (-) | |
| Na | 141 mmol/L | | |
| K | 3.8 mmol/L | | |
| Cl | 104 mmol/L | | |
| CK | 75 U/L | | |
| CRP | 0.28 mg/dL | | |
| LDH | 187 U/L | | |

**Note:** WBC: White Blood Cells; ATL: Adult T-cell Leukemia; RBC: Red Blood Cells; Hb: Hemoglobin; Plt: Platelets; Alb: Albumin; BUN: Blood Urea Nitrogen; AST: Aspartate Transaminase; ALT: Alanine Transaminase; ALP: Alanine Phosphatase; CRP: C-Reactive Protein; LDH: Lactate Dehydrogenase; γGTP: Gamma-glutyl-Transpeptidase.

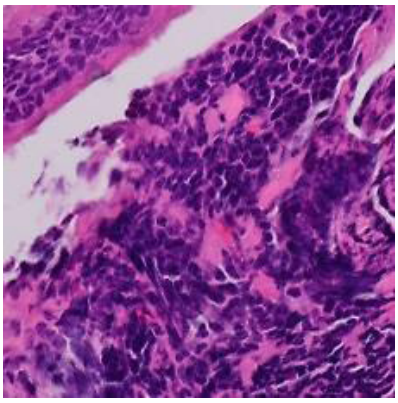

**Figure 3.** Bronchial mucosal findings obtained by bronchoscopy.

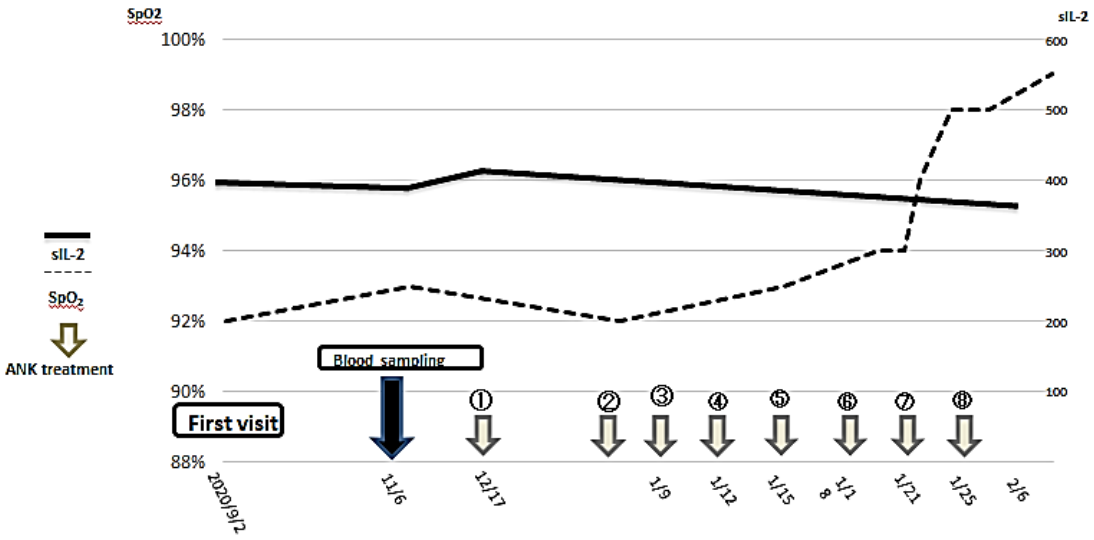


**Figure 4.** Treatment schedule and changes in SpO2 and soluble interleukin 2 (sIL2). **Note:** ( ▬ ) sIL-2; ( - - - - ) $SpO_2$; ( ⇩ ) ANK treatment.

**Table 2.** Pulmonary function tests.

| | Before treatment | | After treatment | |
|---|---|---|---|---|
| | Measured value | % Predicted value | Measured value | % Predicted value |
| VC | 0.93 L | 46.5 | 1.54 L | 78.6 |
| FVC | 0.88 L | 44 | 1.18 L | 60.2 |
| FEV1.0 | 0.83 L | 57.6 | 1.12 L | 82.4 |
| FEV1.0% | 94.30% | 127 | 94.90% | 128.5 |

**Note:** VC: Vital Capacity; FEV: Forced Vital Capacity; FEV: Forced Expiratory volume.

## Cases of cancer

**Case 3:** The patient is a 75-year-old man. In May 2011 he was diagnosed with prostate cancer, bone metastases. Hormone therapy was started in June, and PSA was temporarily improved. CT and bone scintigraphy in July 2014 showed that multiple bone metastases were exacerbating, so in September 2014, we decided to start ANK therapy and collected 12 doses of NK cells. Bone scintigraphy showed improvement in all bone metastases, and PSA was normal. Hormone therapy alone was continued, but in August 2015, hormonal therapy was also discontinued because no recurrence was observed. After 5 years, the patient has been stable without recurrence (Figure 6).

**Case 4:** The patient is a 71 year old female woman. In June 2012, she was diagnosed with stage 4 left breast cancer, multiple lymph node metastases, lung metastases, liver metastases, and bone metastases. She had a CEA of 214. She was being treated with Herceptin and paclitaxel. Although her metastases were generally improving, she had metastases in the second cervical vertebrae and atlantoaxial subluxation, so she was advised to receive ANK therapy.

In October 2012, she performed 6 ANK collections as the 1st time. She then underwent 12 treatments during January-March 2013, diluting her to half her normal dose. After the treatment, metastatic lesions tended to shrink overall, and CEA also showed a downward trend. In August 2013, she performed 6 ANK collections as the 2nd time. Treatment was similar to her first. CEA improved to normal at the end of her treatment. In July 2018, she underwent her left mastectomy for local control. She performed 12 ANK collections as the 3rd time in April 2022 because her CEA rose again to 14.4. She was treated by May-June. Tumor markers became negative again, and metastases were controlled (Figure 7).

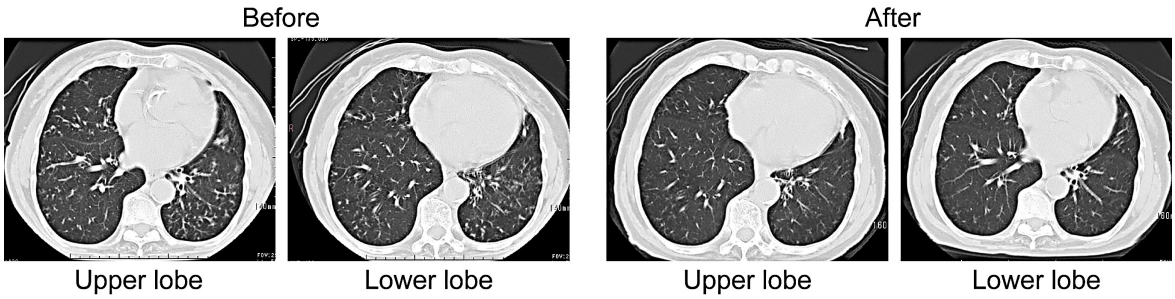


**Figure 5.** Chest CT findings before and after ANK.

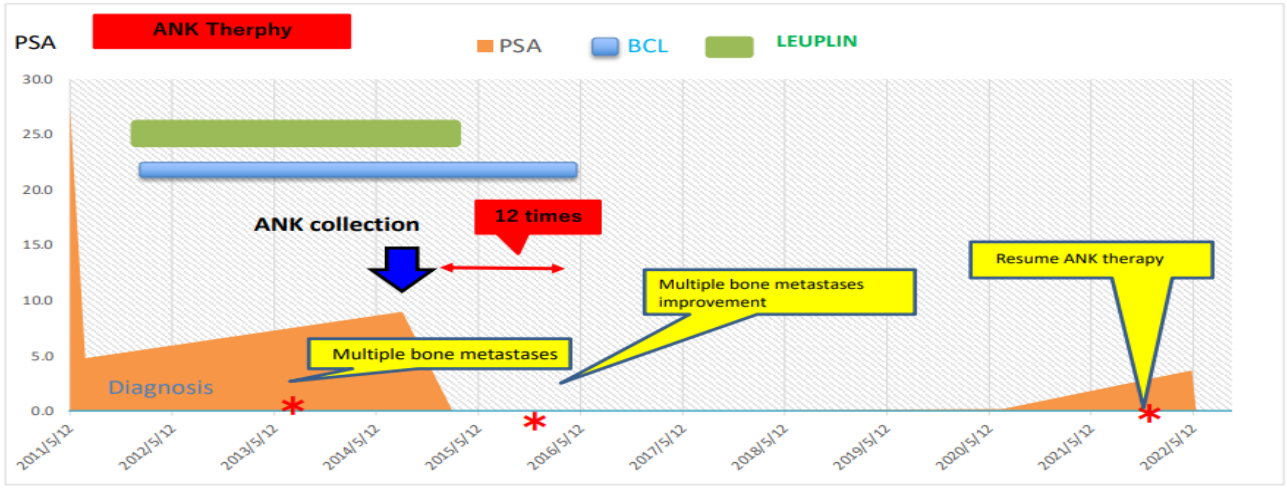


**Figure 6.** Prostate cancer treatment progress and changes in PSA. **Note:** (■) PSA; (■) BCL; (■) LEUPLIN.

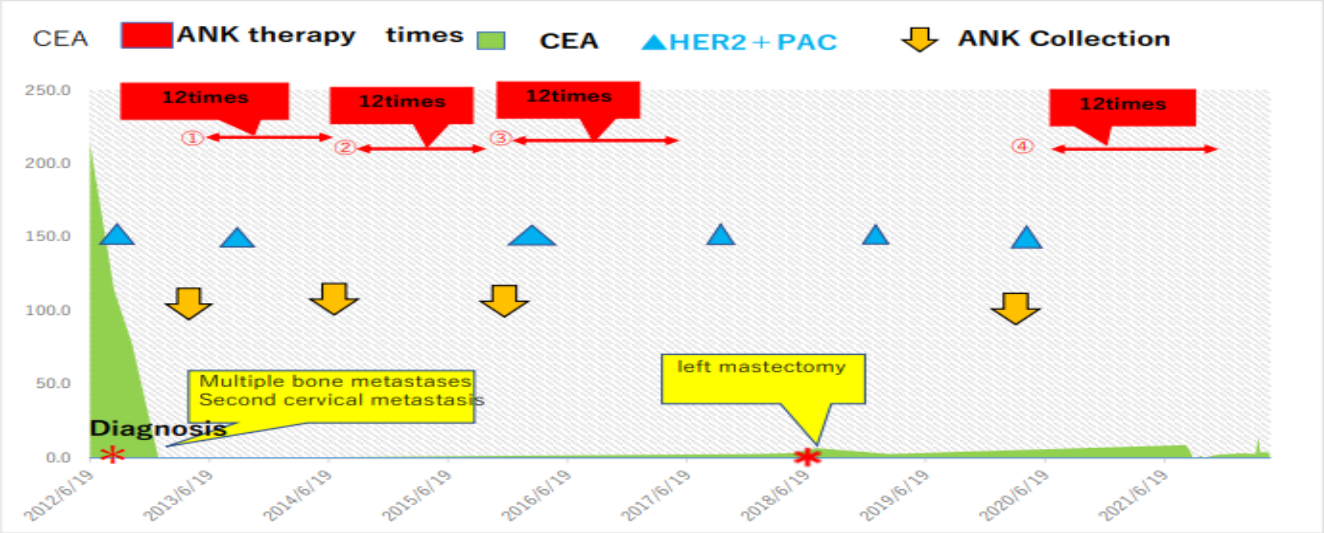


**Figure 7.** Breast cancer treatment progress and changes in CEA. **Note:** (■) ANK therapy times; (■) CEA; (▲) HER2+PAC; (🡻) ANK Collection.

## Results and Discussion

The ANK therapy used this time is completely different from the conventional immunotherapy for which the lymphocyte bank has technology, and it is involved in the production of ANK cells. ANK therapy is a treatment method in which approximately 5 L of blood is collected from the patient; the activated NK cells are amplified, and returned to the patient. For detailed culture and production methods of ANK cells, please refer to the papers [8,9]. The reason his ANK therapy was effective in these two cases is that ATL leukemia cells express NK cell co-stimulatory molecules such as CD80 and CD137L. ATL cells have regulatory T cell characteristics, and ATL patients are immunosuppressed. From this, it is clear that ATL cells express both stimulatory cofactors and inhibitory factors such as PD-L1. It has been reported that NK cells, which are extracted from blood, cultured and activated, can attack tumors regardless of tumor suppressor gene expression, unlike T cells [10].

For this reason, ANK therapy is specific to tumor cells and carries a low risk of significant damage to the normal immune system. With this treatment, patients with ATL may undergo anti-CCR4 [11,12] or anti-PD-1 therapy do not experience the serious side effects seen in [13]. High expression of PD-1 in ATL cells has also been reported [14]. Therefore, anti-PD-1 antibodies may inhibit PD-1-and PD-L1-mediated negative auto regulatory signals in ATL cells and induce proliferation of ATL cells. In ATL, PD-L1 may function both in her ATL cell proliferation and as an immunosuppressant of the normal immune system. It is important to suppress PD-L1. A large number of ANK cells have even been reported to kill PD-L1-positive tumor cells [15]. These findings suggest that repeated administration of NK cells, including ANK cells, relieves immunosuppression through the PD-1-PD-L1 pathway. The onset of clinical outcomes in ATL often occurs only after a long latency period and continues into old age. The percentage of NK cells with the CD16+CD56+phenotype is known to be significantly lower in HTLV-1 infected carriers. Therefore, administering activated NK cells in the form of ANK therapy is considered effective [16].

The mechanisms of lung lesions induced by HTLV-1 in Case 2 include 1) her G1 (proliferative) arrest of her T cells by the viral tax gene, 2) anti-apoptotic effects by NF-κB activation, or 3) p53. DNA damage repair by inhibition. HTLV-1 primarily infects CD4+ T lymphocytes and alveolar epithelium, activates NF-κB, and causes HTLV-1-associated lung disease [17-19].

In addition, other cancers with genomic alterations similar to ATL are common, such as diffuse large cell lymphoma, gastric, esophageal and cervical cancers. , ANK therapy for ATL is considered to be highly effective. There are cases in which ANK therapy is very effective, such as the two cases of solid tumors reported this time. This suggests that ANK therapy killed her PD-L1-positive tumor cells and may be highly effective against cancers with high PD-L1 expression. For solid tumors, PD-L1 levels can be considered a biomarker to predict treatment efficacy. By narrowing down target cancers using biomarkers, it may be possible to provide treatments that are more effective and safer than existing anticancer drugs and immunotherapy treatments. Further studies are needed to prove this hypothesis.

## Conclusion

ANK therapy is completely different from existing immunotherapies. By amplifying the activated NK cells, it is possible to attack without narrowing down the target, and the side effects are minor. In particular, ATL cannot be expected to be effective with existing treatments, and there are many side effects that make treatment difficult. ANK therapy has been found to be effective through two mechanisms that suppress PD-L1, and previous studies have shown that if the cancer has many PD-L1-positive tumor cells, even solid tumors are highly susceptible. It has been suggested that it may be effective.

Based on these reports, ANK therapy is more effective and safer than existing treatments by choosing ANK therapy as the first treatment for solid tumors as well as for ATL. Patients who have given up on treatment may have a chance to get treatment.

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## Declaration of Conflicting Interests

The authors have no conflicts of interest to declare.

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