

## CASE REPORT

# Long-term (>5-year) survivors among unresectable or metastatic cancer patients treated with zoledronate-activated killer cells: Seven case reports

Yoshiyuki Yamaguchi 

Department of Clinical Oncology,  
Kawasaki Medical School and Hospital,  
Kurashiki, Japan

**Correspondence**

Yoshiyuki Yamaguchi, Department of  
Clinical Oncology, Kawasaki Medical  
School and Hospital, Matsushima 577,  
Kurashiki, Okayama 701-0192, Japan.  
Email: [shogo@med.kawasaki-m.ac.jp](mailto:shogo@med.kawasaki-m.ac.jp)

**Abstract**

We are conducting an adoptive immunotherapy (AIT) trial using zoledronate-activated killer cells for patients in Japan with incurable cancer. We have encountered seven long-term survivors. Since such long survival may be very rare in cancer treatment, we present the details and a discussion of a possible role of AIT.

**KEYWORDS**

adoptive immunotherapy, cancer, survivors, Zoledronate-activated killer cells

## 1 | INTRODUCTION

The discovery and molecular cloning of the crucial lymphocyte growth factor interleukin-2 (IL-2) has facilitated the clinical application of adoptive immunotherapy (AIT) for cancer treatments using autologous lymphocytes that are activated *ex vivo* with IL-2.<sup>1</sup> We have been conducting an AIT trial toward the development of novel cancer treatments that use activated autologous lymphocytes.<sup>2</sup> Since 2009, we have introduced the use of zoledronate-activated killer (ZAK) cells into the AIT trial.<sup>3</sup> To date, more than 600 patients with various cancer types have been treated with ZAK-cell AIT.<sup>4,5</sup> Here, we summarize the cases of seven long-term (>5-year) survivors among the trial's patients with unresectable or metastatic cancer treated with ZAK-cell AIT. We also discuss a possible role of ZAK-cell AIT for cancer treatment.

## 2 | PATIENTS AND METHODS

### 2.1 | Patients

All seven of the patients were Japanese and had a diagnosis of unresectable or metastatic cancer with a performance status that allowed them to visit our outpatient clinic. All

patients provided written informed consent for their cases and images to be published. The exclusion criteria were as follows: consecutive use of steroids or immunosuppressants, concurrent use of immune-checkpoint inhibitors, the presence of autoimmune disease, a case that was too difficult to manage at an outpatient clinic, and/or uncontrolled complications. Patients were treated with AIT until their death, their withdrawal of consent for treatment, or the loss of follow-up contact. All aspects of the patients' treatments over time, including specific chemotherapy agents and/or combinations, as well as the dose, schedule, and duration of AIT were determined by a physician on a case-by-case basis. The objective tumor response was evaluated by computed tomography (CT) examinations every 2–3 months by a physician and by measurements of the serum levels of tumor markers. This series of case report was reviewed and approved by the Research Ethics Committee of Kawasaki Medical School and Hospital (approval no. 5264).

### 2.2 | ZAK-cell generation and AIT

The generation of ZAK cells has been described in detail.<sup>6</sup> ZAK cells were administered intravenously for 30 min every

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2022 The Author. *Clinical Case Reports* published by John Wiley & Sons Ltd.

3–4 weeks in a chemotherapy-off period. At each infusion, the patient had blood drawn for the preparation of ZAK cells for the next transfer. Phenotyping for the CD3, CD56, and  $\gamma\delta$ -type T-cell receptor expressions of generated ZAK cells was performed using flowcytometry for the quality assessment of ZAK cells. Bacterial, endotoxin, and mycoplasma examinations were completed before each administration of ZAK cells to ensure that there was no contamination.

### 2.3 | Approval of AIT

AIT using ZAK cells has been provided at Kawasaki Medical School Hospital from May 2009 and was approved by Japan's government in October 2009. This AIT treatment was reviewed for its science and ethics and approved in November 2015 by the Certified Committee for the Regenerative Medicine of Kawasaki Medical School and Hospital (Committee no. NB6150002; protocol no. PC6150017; Japan Registry of Clinical Trials [jRCT] number, jRCTc060190032). Data sets for this case report were fixed in May 2021.

## 3 | CASE PRESENTATION

### 3.1 | Case 1

A 61-year-old male patient diagnosed with intrahepatic cholangiocarcinoma (CCA) has been treated with ZAK-cell AIT for 10 years and 9 months as of this writing. He had undergone a hepatic surgery at his primary-care hospital; 3 years later, he underwent a vertebral surgery for bone metastasis (Th3), which was histologically confirmed with the surgical specimen. Since lung metastasis was indicated 1 year later (Figure 1A), chemotherapy with oral S-1 was started in July 2010 in combination with our ZAK-cell AIT initially every 3 weeks (Figure 1B). At the baseline of the ZAK-cell AIT, the patient's neutrophil-to-lymphocyte ratio (NLR) was 1.7. His serum carbohydrate antigen (CA) 19–9 level seemed to increase during the treatment's initial 8 months but began to decrease thereafter to the normal range. In July 2011, a CT examination showed no lung metastasis and no new lesions. The oral S-1 was discontinued in March 2012, but the ZAK-cell AIT was continued thereafter based on the patient's wishes. He has received a ZAK-cell administration 78 times, the total cell number of which is  $70.9 \times 10^8$  cells. He is alive as of this writing with a normal CA19-9 level and no evidence of clinical recurrence.

### 3.2 | Case 2

A 33-year-old male patient diagnosed with peritoneal metastasis from gastric cancer has been treated with ZAK-cell

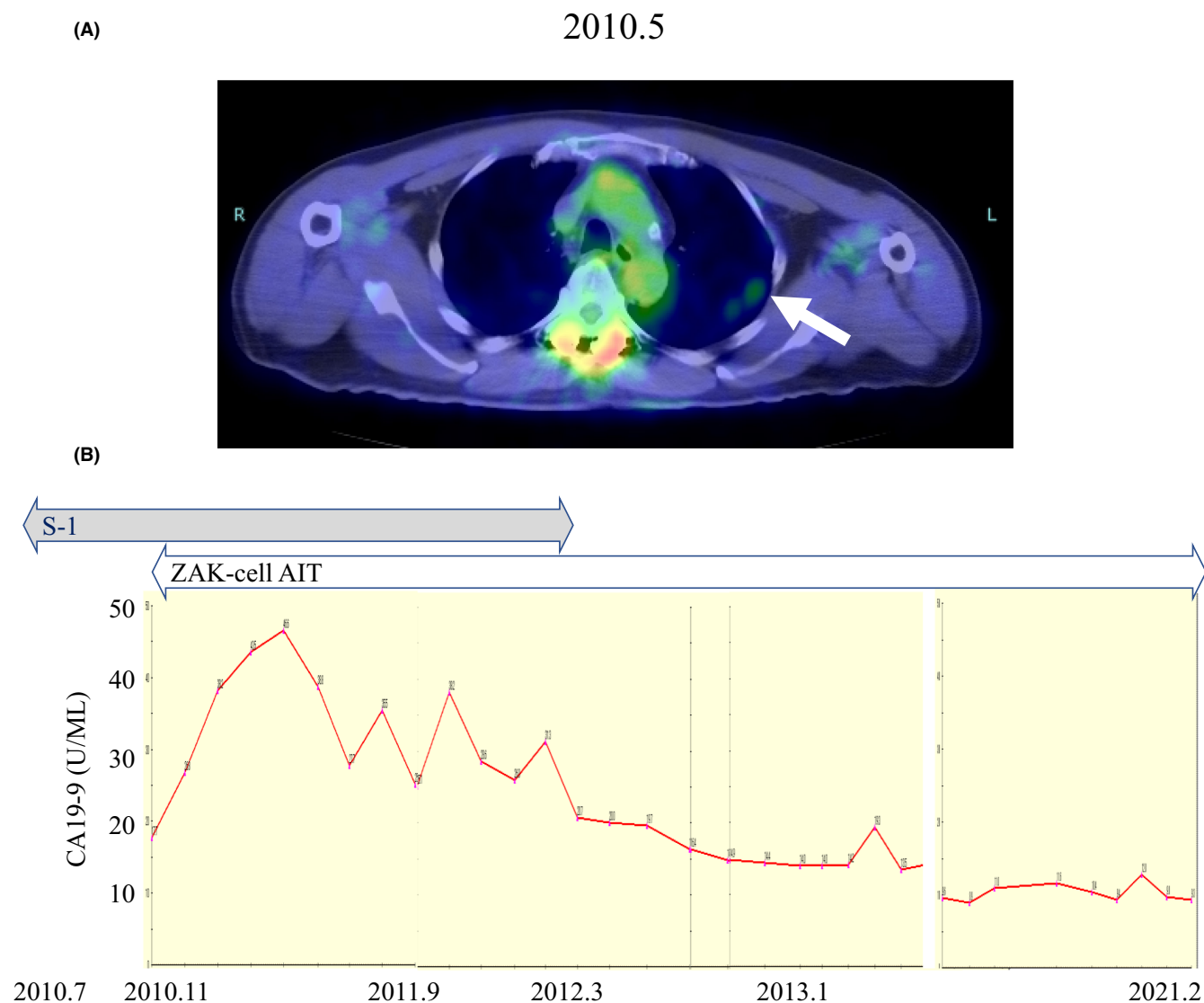
AIT for 9 years and 4 months. In April 2010, he underwent a total gastrectomy with lymphadenectomy and was at high risk of recurrence of stage III disease. Two months later, a positron emission tomography (PET) examination indicated the accumulation of glucose at the Douglas portion with an abnormal CA19-9 level. Chemotherapy using S-1 plus cisplatin was administered from November 2010 for 2 years. Beginning in December 2011, ZAK-cell AIT was added to the chemotherapy, initially every 3 weeks. At the baseline of the AIT, the patient's NLR was 0.3. His serum CA19-9 level decreased after the combination treatment. The ZAK-cell AIT was continued every 2 months after the cessation of the chemotherapy on the patient's wishes. As of this writing, the patient has received 88 ZAK-cell administrations, the total cell number of which is  $87.7 \times 10^8$  cells. He is alive with an abnormal but stable CA19-9 level.

### 3.3 | Case 3

A 42-year-old female patient diagnosed with renal cell carcinoma (RCC) with lung metastasis has been treated with ZAK-cell AIT for 7 years and 8 months. She had undergone a nephrectomy in April 2014. Chemotherapy with sunitinib was administered for lung metastasis just after that surgery (Figure 2A), and in September 2014 a CT examination revealed shrinkage of the metastases. The chemotherapy was stopped in May 2015 due to an adverse event, that is, secondary nephrotic syndrome, when a CT examination showed no lung metastasis (Figure 2B). ZAK-cell AIT was started in August 2015 initially every 3 weeks; at baseline, the patient's NLR was 6.2. The patient has received 43 administrations of ZAK-cell AIT, the total cell number of which is  $23.4 \times 10^8$  cells. She is alive with no detectable lung metastases.

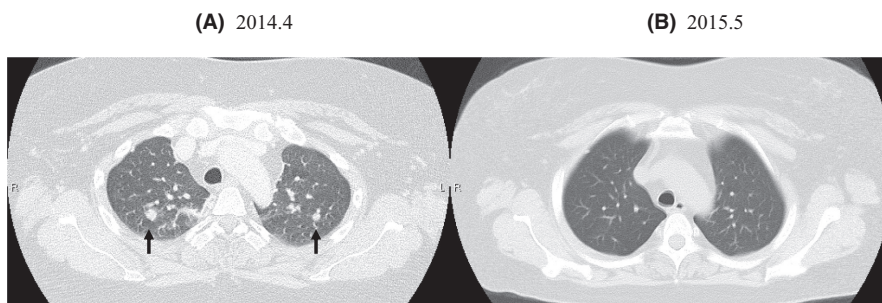
### 3.4 | Case 4

A 72-year-old male patient diagnosed with pancreatic cancer with celiac artery invasion (Figure 3A) has been treated with ZAK-cell AIT for 5 years and 5 months. In March 2015, he had received radiotherapy in combination with chemotherapy using S-1. In August 2015, a CT examination revealed shrinkage of the primary tumor (Figure 3B). Beginning in November 2015, ZAK-cell AIT was initiated over the S-1 chemotherapy, initially every 3 weeks. The patient's NLR was 3.9 at the AIT baseline. His serum carcinoembryonic antigen (CEA) level decreased after the combination treatment, reaching the normal range in February 2016. The ZAK-cell AIT was discontinued in March 2017 in accord with his wishes. However, a later increase in his CEA level led him to decide to resume the ZAK-cell AIT. The CEA level decreased



**FIGURE 1** Clinical course of the patient with CCA (case No. 1). (A), a PET-CT image of lung metastasis (arrow) before ZAK-cell AIT; (B), changes in the level of CA 19-9 were normalized under the period of chemotherapy S-1 plus ZAK-cell AIT.

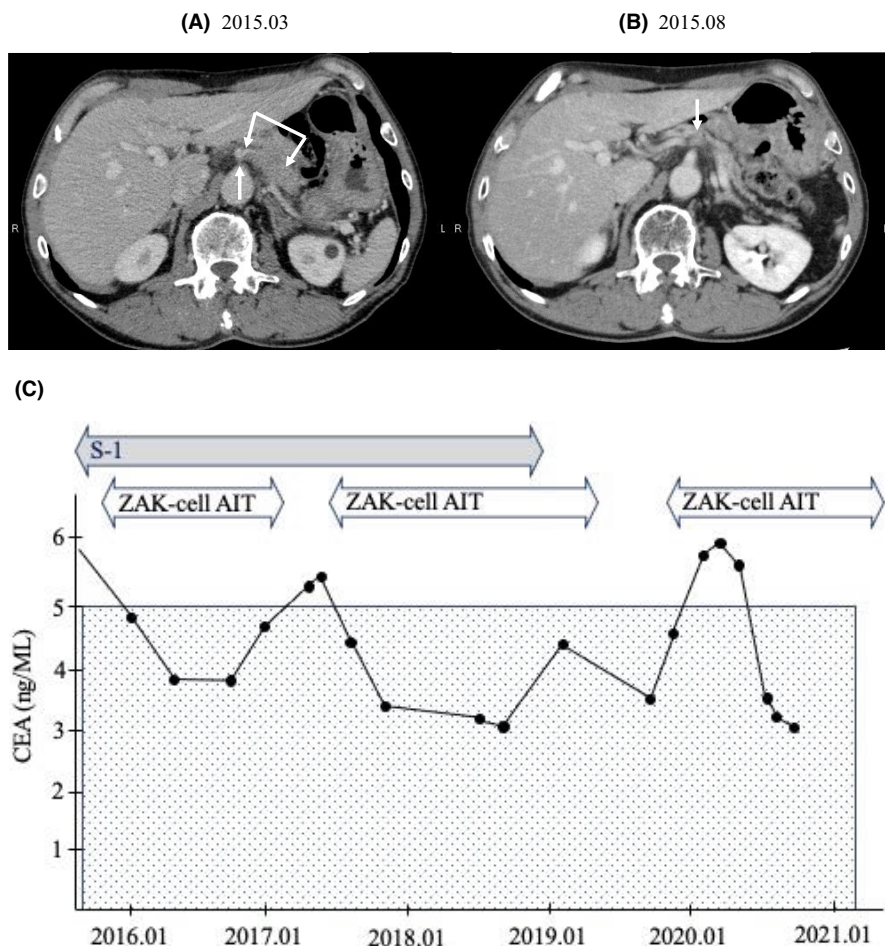
**FIGURE 2** CT images of the patient with RCC (Case No. 3). CT images of the patient with RCC before (A) and after (B) chemotherapy. The lung metastasis observed in panel (A) disappeared after the treatment (B).



thereafter to within a normal range, again (Figure 3C). The S-1 treatment was discontinued in November 2018. He has received 39 ZAK-cell administrations, the total cell number of which is  $21.4 \times 10^8$  cells, and CT showed that his pancreas was stable. He is alive with a normal CEA level.

### 3.5 | Case 5

A 43-year-old female patient diagnosed with breast cancer with lung metastasis has been treated with ZAK-cell AIT for 5 years and 3 months. She had undergone breast surgery and a lymphadenectomy, followed by adjuvant



**FIGURE 3** CT images and CEA evaluation of the patient with pancreatic cancer (Case No. 4). CT images of the patient with pancreatic cancer before (A) and after (B) chemoradiotherapy plus ZAK-cell AIT. The tumor at the pancreas body (combined arrow) with celiac artery invasion (arrow) observed in (A) shrank along with a normalized CEA value (arrow) after the treatment (B). Changes in the level of CEA are shown (Figure 3C). Dotted area indicates the normal range of CEA.

hormone therapy. Two years later, she underwent pulmonary surgery for a solitary lung tumor, which was histologically diagnosed as lung metastasis from breast cancer. Eighteen months later, lung metastasis appeared again. Chemotherapy with anastrozole was initiated in December 2015. In January 2016, ZAK-cell AIT was added to the hormone therapy, initially every 3 weeks. The NLR was 1.1 at baseline. In April 2016, a CT examination revealed shrinkage of part of the metastases, and a complete remission was confirmed in April 2017. The ZAK-cell AIT has continued every 3 months in combination with the hormone therapy in accord with the patient's wishes. She has received 33 ZAK-cell administrations, the total cell number of which is  $17.4 \times 10^8$  cells. She is alive with no detectable lung metastases.

### 3.6 | Case 6

A 51-year-old female patient diagnosed with ovarian squamous cell carcinoma (SCC) with lung and liver metastasis has been treated with ZAK-cell AIT for 5 years. In January 2016, she had undergone a salpingo-oophorectomy with lymphadenectomy. Chemotherapy

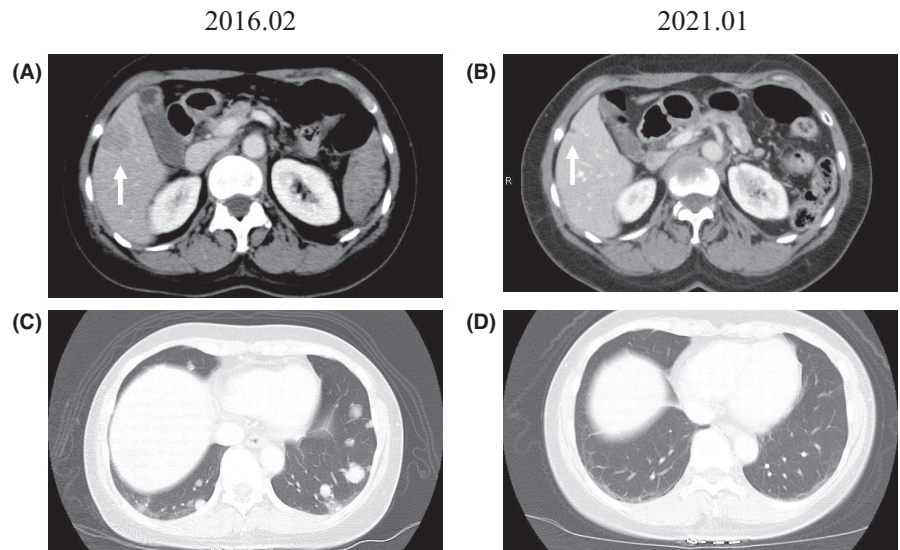
using paclitaxel plus carboplatin was administered for lung and liver metastasis from February 2016 for 1 year (Figure 4A,C). In April 2016, ZAK-cell AIT was added to the chemotherapy, initially every 3 weeks. The patient's NLR was 0.7 at the AIT baseline. Her serum SCC-related antigen level decreased to the normal range after the combination treatment, and in parallel, a CT examination revealed shrinkage of the metastases in May 2016. The ZAK-cell AIT every 3 weeks has been continued after the cessation of the chemotherapy per the patient's wishes. She has received 51 ZAK-cell administrations, the total cell number of which is  $26.6 \times 10^8$  cells. She is alive with a normal SCC-related antigen level, a marginal shadow of liver metastasis, and no lung metastases (Figure 4B,D).

### 3.7 | Case 7

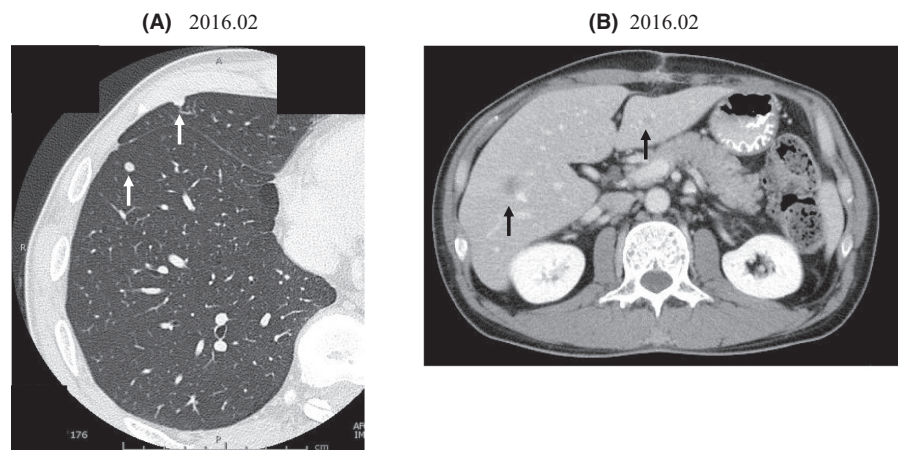
A 53-year-old male patient diagnosed with colon cancer with liver and lung metastases has been treated with ZAK-cell AIT for 5 years. In January 2016, he had undergone a colectomy with lymphadenectomy followed by oxaliplatin-based chemotherapy plus bevacizumab (Figure 5A,B).



**FIGURE 4** CT images of the patient with ovarian cancer (Case No. 6). CT images of the patient with ovarian cancer before (A, C) and after (B, D) chemotherapy plus ZAK-cell AIT. The patient's liver metastasis (arrow) shrank to a trace extent and lung metastases disappeared with normalized serum SCC values after the treatment.



**FIGURE 5** CT images of the patient with colon cancer (Case No. 7). The arrows indicate lung metastasis (A) and liver metastasis (B) before ZAK-cell AIT.



Beginning in April 2016, ZAK-cell AIT initially every 3 weeks was administered in addition to the chemotherapy. His NLR was 2.2 at the AIT baseline. His serum CEA level decreased but fluctuated between abnormal and normal values. He received multiple lines of chemotherapy, liver surgery, and radiofrequency ablation for lung metastases in combination with ZAK-cell AIT. He has received 46 administrations of ZAK cells, the total cell number of which is  $26.3 \times 10^8$  cells. He is alive with liver and lung metastases and an abnormal but stable CEA level.

The details of these seven patients' cases are summarized in Table 1. All patients received concurrent chemotherapy with ZAK-cell AIT, which was eventually discontinued in five of the seven patients; the other two patients are receiving hormone therapy for breast cancer and chemotherapy for colon cancer, respectively. Tumor markers were available in five cases and were normalized for a long term in three of the patients (one each with CCA, pancreatic cancer, and ovarian cancer). At present, residual tumors are evident in four patients, as indicated by CT imaging and/or abnormal tumor marker

levels. The NLR values were  $<3$  in all but two of the patients, who had RCC and pancreatic cancer, respectively. ZAK-cell transfer was conducted 33 to 88 times in the seven patients, and the transferred cell numbers varied between  $17.4$  and  $87.7 \times 10^8$  cells in total, at  $0.5$  and  $1.0 \times 10^8$  cells per transfer. The lymphocyte phenotype of ZAK cells showed that the  $\gamma\delta T$  phenotype was predominant compared with the CD56 phenotype in two patients (the RCC and ovarian cancer patients), while the CD56 phenotype was predominant in the other five patients.

## 4 | DISCUSSION

In our ZAK-cell AIT trial with over 600 patients,<sup>3</sup> we have encountered seven long-term ( $>5$ -year) survivors with unresectable or metastatic solid tumors. In general, the only treatment modality to cure solid tumors is surgery, and survival for  $>5$  years is quite rare among patients with unresectable or metastatic solid tumors, even when treated

TABLE 1 Summary of long-term (&gt;5-year) survivors treated with ZAK-cell AIT

Case No.	Age	Gender	Disease	Metastasis	Histological definition for metastasis (site)	Combination treatment (period/response)
1	61	Male	Intrahepatic cholangiocarcinoma	Lung, bone	yes (bone)	S-1 (1 year 8 months/CR)
2	33	Male	Gastric cancer	Peritoneum	none	S-1 + CDDP (2 years/non-CR non-PD)
3	42	Female	Renal cell carcinoma	Lung	none	Sunitinib (1 year/CR)
4	72	Male	Pancreatic cancer	SMA invasion	none	RT + S-1 (3 years/non-CR non-PD)
5	43	Female	Breast cancer	Lung	yes (lung)	Hormone (5 years/CR)
6	51	Female	Ovarian cancer	Lung, Liver	none	Carboplatin+Paclitaxel (1 year/PR)
7	53	Male	Colon cancer	Liver, lung	yes (liver)	Bevacizumab+FU + Oxaliplatin/CPT-11 + surgery+ablation (5 years/SD)

Abbreviation: NE, no evidence of tumor.

with anticancer chemotherapeutic drugs. Our above-described CCA patient with histologically confirmed bone metastasis (Case No. 1) has remained alive for >10 years after ZAK cell AIT. He had been treated with chemotherapy using S-1, and his long survival could be due to the S-1 chemotherapy. The S-1 treatment was provided only during the initial 18 months with the elevated CA19-9 remaining, and the patient was treated with ZAK-cell AIT alone during most of the treatment period. In a phase II study of S-1 treatment for patients with unresectable biliary tract cancer, the objective response rate (OR) was 21.1% including no complete response (CR), and the median overall survival (OS) period was 8.3 months.<sup>7</sup> In patients with unresectable biliary tract cancer treated with fluorouracil-based regimens, the reported OR was 0%–34% and the OS period was 4.5–14.8 months.<sup>8</sup> This suggests that in our patient with CCA (Case No. 1), it cannot be concluded that only the S-1 treatment was responsible for the long-term survival.

Our above-described patient with metastatic RCC (Case No. 3) has remained alive for >7 years after ZAK-cell AIT. In a phase III randomized trial of sunitinib versus interferon- $\alpha$  (IFN- $\alpha$ ) as the first-line systemic therapy for patients with metastatic RCC, it was reported that the OR was 47% including a 3% CR rate for sunitinib.<sup>9</sup> This may indicate the efficacy of sunitinib in our RCC patient (Case No. 3). However, it was also reported in that trial<sup>9</sup> that the median progression-free survival (PFS) and OS periods of the sunitinib-treated patients were 11 and 26.4 months, respectively. Given these findings, it also seems unlikely that our RCC patient's long-term survival is due only to her sunitinib treatment.

Our patient with metastatic ovarian SCC (Case No. 6) has survived 5 years after ZAK-cell AIT. Ovarian cancer

is known as a chemotherapy-sensitive tumor. According to the data of a randomized controlled trial comparing dose-dense paclitaxel in combination with carboplatin for advanced ovarian cancer, the OR rate was approx. 70% including approx. 20% CRs,<sup>10</sup> and the 5-year OS rate was ~55%.<sup>11</sup> This suggests that the long-term survival of our patient may be due to her chemotherapy using paclitaxel plus carboplatin. However, ovarian SCC is a rare disease that is known to generally have a poor prognosis compared with adenocarcinomas.<sup>12</sup> Taking the above information together, it also seems unlikely that the long-term survival with an almost complete response in our patient with metastatic ovarian SCC was afforded by only the chemotherapy.

The other four patients in our present report (with gastric, pancreatic, breast, and colon cancer) have achieved >5-year survival with their tumors after ZAK-cell AIT. It is likely that the multidisciplinary treatments in these patients are responsible for the long-term survival. It may not be rare that hormone-sensitive breast cancer and chemotherapy-sensitive colon cancer show durable responses to hormone therapy and chemotherapy, respectively; however, gastric cancer with peritoneal metastasis is usually incurable. In addition, in the patient with pancreatic cancer, the tumor marker level increased after the cessation of ZAK-cell AIT and recovered after the resumption of ZAK-cell AIT, indicating clearly the tumor-controlling effect of ZAK-cell AIT. Schreiber et al.<sup>13</sup> proposed the “cancer immunoediting” theory, which proceeds sequentially through three distinct phases termed “elimination,” “equilibrium,” and “escape”. We speculate that ZAK-cell AIT may have contributed to building the equilibrium phase in our four patients.

Tumor marker (response)	Present residual tumor (evidence)	Survival time after ZAK-cell AIT (years and months)	NLR at baseline	ZAK-cell AIT, Total cell No. ( $\times 10^8$ )/No. of transfer/mean No. ( $\times 10^8$ )	Phenotype of ZAK cells (mean, range)		
					CD3	$\gamma\delta$ T	CD56
CA19-9 (normalized)	NE	10years 9 months	1.7	70.9/78/0.9	56 (12–83)	2 (1–6)	77 (55–96)
CA19-9 (abnormal)	yes (CA19-9)	9 years 4 months	0.3	87.7/88/1.0	36 (9–76)	13 (4–29)	86 (30–95)
none	NE	7years 8 months	6.2	23.4/43/0.5	79 (17–92)	67 (16–91)	47 (20–78)
CEA (normalized)	yes (CT)	5 years 5 months	3.9	21.4/39/0.5	43 (10–65)	18 (5–42)	69 (13–86)
none	NE	5 years 3 months	1.1	17.4/33/0.5	64 (39–92)	40 (15–56)	65 (37–83)
SCC (normalized)	yes (CT)	5 years	0.7	26.6/51/0.5	73 (33–92)	56 (14–80)	54 (30–84)
CEA (abnormal)	yes (CT, CEA)	5 years	2.2	26.3/46/0.6	50 (10–92)	12 (1–29)	88 (22–98)

We propose that ZAK-cell AIT has played an important and effective role in the treatment of our seven patients. Our ZAK cells have consisted of natural killer (NK) cells and  $\gamma\delta$ T cells, as in our previous report.<sup>6</sup> NK cells have been reported to demonstrate anti-tumor cytotoxicity (without prior sensitization) and the production of cytokines as well as chemokines that regulate various immune responses.<sup>14</sup> It was also demonstrated that  $\gamma\delta$ T cells had the ability to kill a wide variety of tumor cells, and that these cells played an important role in the innate immune system.<sup>15</sup> Moreover,  $\gamma\delta$ T cells have been shown to possess an antigen-presenting function.<sup>16</sup> Intra-tumoral  $\gamma\delta$ T-cell signatures have emerged as the most significant favorable cancer-wide prognostic populations, and it was noted that  $\gamma\delta$ T-cell and CD8 T-cell signatures were the most highly correlated, suggesting a link to the prognostic significance of these cells.<sup>17</sup> These findings suggest that ZAK-cell AIT may make a positive contribution to patients' immunological status in their multidisciplinary cancer treatment.

In the field of lung cancer, Gettinger et al.<sup>18</sup> observed long-term survivals and durable responses in a proportion of patients with pretreated advanced non-small cell lung cancer in the 5-year follow-up results from an early phase I study of the anti-programmed death-1 antibody nivolumab. A marked shift recently occurred in lung cancer treatment, in which treatment with chemotherapy plus an immune-checkpoint inhibitor has been established as a standard treatment.<sup>19</sup> The only exception to long-term survival due to treatment with cytotoxic chemotherapeutic drugs, if any, may thus be cases involving the emergence of effective anti-tumor immune responses during chemotherapy.

The existence of a cancer-immunity cycle has been proposed: a series of stepwise events for an anticancer immune

response that leads to the effective killing of cancer cells, starting from neoantigen-release and its capture for antigen processing by dendritic cells (DCs) (step 1); followed by neoantigen presentation by DCs (step 2); the priming and activation of T cells (step 3); the trafficking (step 4), and infiltration (step 5) of T cells into cancer tissue; the T-cell recognition of neoantigens on tumor cells (step 6); and the killing tumor cells (step 7), then continuing to step 1 in another cycle.<sup>20</sup> This cycle would repeat continuously to eliminate cancer. We speculate that the cytotoxic chemotherapeutic drugs and ZAK cells may trigger the neoantigen-release step of the cancer-immunity cycle followed by the antigen-specific immune activation, and the cancer-immunity cycle may have functioned well in our patients treated with ZAK-cell AIT, resulting in their long-term survival.

It is clear that ZAK-cell AIT has not been effective for all of the cancer patients who have received this treatment. We have observed a significant positive correlation between longer survival and the baseline NLR value in patients with incurable gastric cancer who were treated with ZAK-cell AIT combined with chemotherapy.<sup>5</sup> A high NLR was also identified as one of the factors that predicted patients who might receive less benefit from nivolumab treatment among patients with pretreated advanced gastric or gastroesophageal junction cancer, in whom the early progression of tumors in some patients was of concern.<sup>21</sup> In our seven present patients' cases, the NLR levels were  $<3$  in all but two of the patients (who had RCC and pancreatic cancer, respectively). The development of a biomarker to predict the efficacy of ZAK-cell AIT is urgently necessary in order to establish ZAK-cell AIT as a standard therapy. The NLR might be a candidate biomarker.

## ACKNOWLEDGMENTS

We thank Mrs. Tomomi Yoshimitsu, Mrs. Yukari Minobe, Miss Naoko Okada, Mrs. Sonoko Sakuma, Miss Akiyo Tamura, Miss Yumi Nishiwaki, and Mr. Akihiro Nyuuya (Department of Clinical Oncology, Kawasaki Medical School) for their invaluable assistance with the lymphocyte culture and immunological analysis, and Mrs. Kikue Tokuda (Department of Clinical Oncology, Kawasaki Medical School) for her excellent management of the clinical data.

## FUNDING INFORMATION

No funding was received.

## CONFLICT OF INTEREST

The author has no conflict of interest to declare.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study

## CONSENT

Written informed consent was obtained from the patient to publish this report in accordance with the journal's patient consent policy.

## ORCID

Yoshiyuki Yamaguchi  <https://orcid.org/0000-0001-5895-9044>

## REFERENCES

- Rosenberg SA, Lotze MT, Muul LM, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N Engl J Med*. 1985;313:1485-1492.
- Yamaguchi Y, Okita R, Emi A, et al. Adoptive immunotherapy of cancer using autologous lymphocytes. *Cancer Growth Progression*. 2011;13(2):285-294.
- Okawaki M, Hironaka K, Yamamura M, Yamaguchi Y. Adoptive immunotherapy using autologous lymphocytes activated ex vivo with antigen stimulation for patients with incurable cancer. *Kawasaki Med J*. 2014;40(1):33-39.
- Yamaguchi Y, Katata Y, Okawaki M, Sawaki A, Yamamura M. A prospective observational study of adoptive immunotherapy for cancer using zoledronate-activated killer (ZAK) cells—an analysis for patients with incurable pancreatic cancer. *Anticancer Res*. 2022;42:1181-1187.
- Yamaguchi Y, Katata Y, Sano F, et al. Adoptive immunotherapy for gastric cancer using zoledronate-activated killer cells: a prospective observational study. *Mol Clin Oncol*. 2020;13:55-62.
- Nagamine I, Yamaguchi Y, Ohara M, Ikeda T, Okada M. Induction of gammadelta T cells using zoledronate plus interleukin-2 in patients with metastatic cancer. *Hiroshima J Med Sci*. 2009;58:37-44.
- Ueno H, Okusaka T, Ikeda M, Takezako Y, Morizane C. Phase II study of S-1 in patients with advanced biliary tract cancer. *Br J Cancer*. 2004;91(10):1769-1774.
- Furuse J, Takada T, Miyazaki M, et al. Guidelines for chemotherapy of biliary tract and ampullary carcinomas. *J Hepatobiliary Pancreat Surg*. 2008;15(1):55-62.
- Motzer RJ, Hutson TE, Tomczak P, et al. Overall survival and updated results for sunitinib compared with interferon alfa in patients with metastatic renal cell carcinoma. *J Clin Oncol*. 2009;27:3584-3590.
- Katsumata N, Yasuda M, Takahashi F, et al. Dose-dense paclitaxel once a week in combination with carboplatin every 3 weeks for advanced ovarian cancer: a phase 3, open-label, randomised controlled trial. *Lancet*. 2009;374:1331-1338.
- Katsumata N, Yasuda M, Isonishi S, et al. Long-term results of dose-dense paclitaxel and carboplatin versus conventional paclitaxel and carboplatin for treatment of advanced epithelial ovarian, fallopian tube, or primary peritoneal cancer (JGOG 3016): a randomised, controlled, open-label trial. *Lancet Oncol*. 2013;14:1020-1026.
- Gurung A, Hung T, Morin J, Gilks CB. Molecular abnormalities in ovarian carcinoma: clinical, morphological and therapeutic correlates. *Histopathology*. 2013;62:59-70.
- Schreiber RD, Oldland LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science*. 2011;331:1565-1570.
- Chiossone L, Dumas PY, Vienne M, Vivier E. Natural killer cells and other innate lymphoid cells in cancer. *Nat Rev Immunol*. 2018;18:671-688.
- Braza MS, Klein B. Anti-tumour immunotherapy with Vγ9Vδ2 T lymphocytes: from the bench to the bedside. *Br J Haematol*. 2013;160:123-132.
- Brandes M, Willmann K, Moser B. Professional antigen presentation function by human gammadelta T cells. *Science*. 2005;309:264-268.
- Gentles AJ, Newman AM, Liu CL, et al. The prognostic landscape of genes and infiltrating immune cells across human cancers. *Nat Med*. 2015;21:938-945.
- Gettinger S, Horn L, Jackman D, et al. Five-year follow-up of Nivolumab in previously treated advanced non-small-cell lung cancer: results from the CA209-003 study. *J Clin Oncol*. 2018;36:1675-1684.
- Gandhi L, Rodríguez-Abreu D, Gadgeel S, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *N Engl J Med*. 2018;378:2078-2092.
- Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. *Immunity*. 2013;39:1-10.
- Kang YK, Morita S, Satoh T, et al. Exploration of predictors of benefit from nivolumab monotherapy for patients with pre-treated advanced gastric and gastroesophageal junction cancer: post hoc subanalysis from the ATTRACTION-2 study. *Gastric Cancer*. 2022;25(1):207-217.

**How to cite this article:** Yamaguchi Y. Long-term (>5-year) survivors among unresectable or metastatic cancer patients treated with zoledronate-activated killer cells: Seven case reports. *Clin Case Rep*. 2022;10:e06540. doi:[10.1002/ccr3.6540](https://doi.org/10.1002/ccr3.6540)