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INVARIANT NATURAL KILLER T CELL–BASED IMMUNOTHERAPY USING α-GALACTOSYLCERAMIDE–PULSED CD14⁺ MONOCYTES FOR ADVANCED GALLBLADDER CANCER

Keigo Hanada[1], Katsuyuki Kobayashi[2]

[1] R&D Department, Ambicion Co., Ltd., Tokyo, Japan
[2] KT Clinic (currently affiliated with Yotsuya Medical Cube), Tokyo, Japan

Corresponding author's e-mail: hanada@ambicion.co.jp

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ABSTRACT

Introduction: Gallbladder cancer is a rare and aggressive malignancy. Patients with unresectable or metastatic disease generally have poor outcomes despite standard chemotherapy. Novel immunotherapeutic strategies that enhance antitumor immunity are of considerable clinical interest. Invariant natural killer T (iNKT) cells rapidly produce cytokines and activate immune effector cells, representing a potential target for immunotherapy.

Case description: We report a case of advanced gallbladder cancer treated with gemcitabine chemotherapy in combination with autologous iNKT cell–targeted therapy. Peripheral blood CD14⁺ monocytes were isolated, pulsed in vitro with α-galactosylceramide (α-GalCer), and administered intravenously to activate iNKT cells in vivo. Post-treatment contrast-enhanced computed tomography scan demonstrated shrinkage of multiple liver metastases and reduction in intra-abdominal lymphadenopathy. Liver function markers improved, and interferon-γ–producing cells increased, indicating systemic immune activation. The therapy was well tolerated, with only mild transient fever observed.

Discussion: Although the specific contribution of immunotherapy cannot be definitively separated from concomitant chemotherapy, the temporal association between α-GalCer–pulsed CD14⁺ monocytes (GC-MO) administration, immune activation, and clinical improvement suggests a potential synergistic effect. Activation of iNKT cells may enhance antitumor immunity, contributing to tumour regression and improved hepatic function. This case highlights the feasibility and safety of combining iNKT-targeted therapy with standard chemotherapy in advanced gallbladder cancer.

Conclusion: iNKT cell–targeted immunotherapy using GC-MO may serve as a promising adjunctive strategy for advanced gallbladder cancer. Further studies are warranted to validate its efficacy and define its role in combination with conventional chemotherapy.

KEYWORDS

Gallbladder cancer, invariant natural killer T cells, α-galactosylceramide, CD14⁺ monocytes, immunotherapy

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LEARNING POINTS

- Invariant natural killer T (iNKT) cells play an important role in antitumor immunity by rapidly producing cytokines and activating multiple immune effector cells, including NK cells and cytotoxic T lymphocytes.
- α-Galactosylceramide–pulsed CD14⁺ monocytes can activate iNKT cells and induce systemic immune responses.
- Combination therapy with chemotherapy and iNKT cell–targeted immunotherapy may contribute to tumour regression and immune activation in patients with advanced gallbladder cancer.

INTRODUCTION

Gallbladder cancer (GBC) is a rare but highly aggressive malignancy and the most common cancer of the biliary tract. Its incidence shows marked geographic variation, with relatively high rates reported in parts of Asia and South America, including Japan[1]. Because early-stage disease is often asymptomatic, many patients are diagnosed at an advanced stage when curative resection is no longer feasible. For patients with unresectable or metastatic disease, gemcitabine-based chemotherapy is the standard treatment; however, the overall prognosis remains poor[2].

Invariant natural killer T (iNKT) cells are a distinct subset of lymphocytes that recognize glycolipid antigens presented by CD1d molecules and rapidly produce cytokines such as interferon-γ, thereby activating multiple antitumor immune effector cells, including natural killer cells and cytotoxic T lymphocytes[3]. Previous studies have demonstrated that dendritic cells pulsed with α-galactosylceramide (α-GalCer) can activate endogenous iNKT cells and induce systemic antitumor immune responses in patients with advanced malignancies[3,4].

Clinical trials using α-GalCer–pulsed antigen-presenting cells have shown the feasibility and immunological activity of iNKT cell-based immunotherapy in patients with advanced cancers, including lung cancer[4]. These findings suggest that iNKT cell-targeted immunotherapy may be a potential strategy to enhance antitumor immunity in refractory malignancies.

Herein, we report a case of advanced GBC treated with gemcitabine chemotherapy in combination with iNKT cell-targeted autologous immune cell therapy.

CASE DESCRIPTION

iNKT cell–targeted immunotherapy is an autologous cell-based treatment using CD14-positive monocytes (CD14MO) isolated from the patient's peripheral blood. Monocytes pulsed with α-galactosylceramide (α-GalCer) function as antigen-presenting cells capable of activating endogenous invariant natural killer T (iNKT) cells. These α-GalCer–pulsed monocytes (GC-MO) were manufactured in a certified cell processing center, cryopreserved, and administered intravenously after quality control testing. The treatment protocol was approved by a certified committee for regenerative medicine in accordance with the Act on the Safety of Regenerative Medicine.

A 61-year-old man (height 170 cm, weight 68 kg) presented with obstructive jaundice and was diagnosed with GBC. Radiological evaluation revealed multiple liver metastases and intra-abdominal lymph node metastases. Systemic chemotherapy was planned; however, the patient requested concomitant iNKT cell-based immunotherapy.

The patient received three cycles of GC-MO (8–10 $\times 10^7$ cells per administration). GC-MO was administered twice per cycle: dose 1 (day 0) and dose 2 (day 25) in cycle 1; dose 1 (day 138) and dose 2 (day 166) in cycle 2; and dose 1 (day 244) and dose 2 (day 272) in cycle 3. Gemcitabine was selected as concomitant chemotherapy at the patient's request and was administered at a reduced

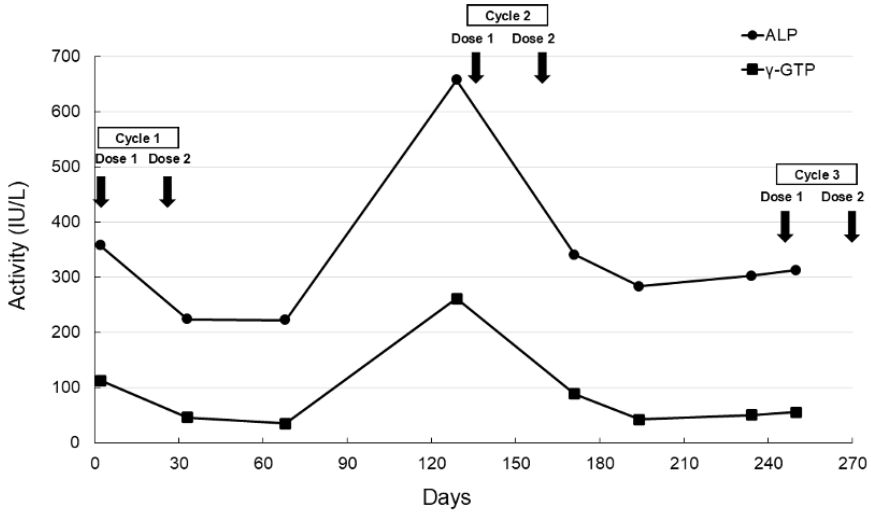


Figure 1. Temporal changes in liver function markers. Serial changes in alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (γ-GTP) levels during treatment are shown. Arrows indicate the timing of dose 1 and dose 2 administrations of α-galactosylceramide–pulsed CD14⁺ monocytes in each treatment cycle.

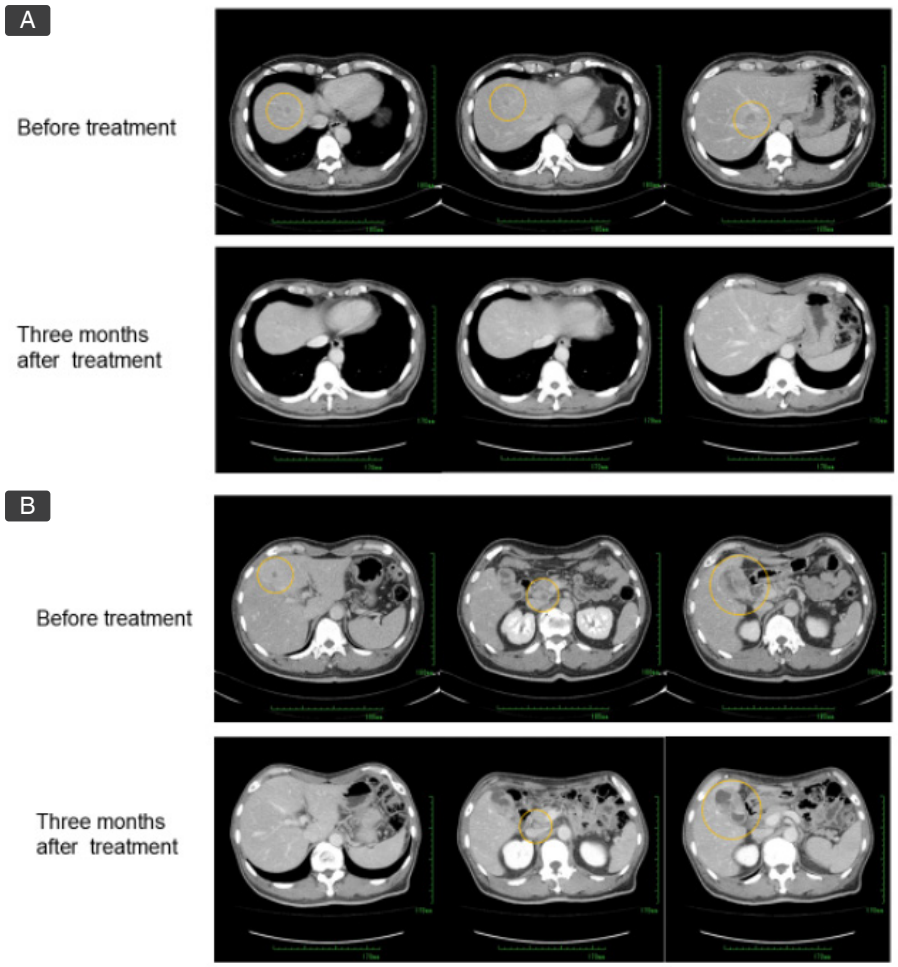


Figure 2. *Contrast-enhanced abdominal computed tomography scan before treatment and at three months after therapy. A) Cross section 1 showing metastatic liver lesions (circled) before treatment and regression at three months; B) Cross section 2 showing intra-abdominal lymph node metastases (circled) before treatment and reduction at three months.*

dose (below the standard 1000 mg/m^2) from day 2 to day 104, followed by a reduction to approximately half of the preceding dose until day 225. In addition, the patient received the Japanese herbal medicine Juzentaihoto (7.5 g/day) prescribed through a Kampo outpatient clinic.

After completion of cycle 1, liver function tests showed decreases in alkaline phosphatase and γ-glutamyl transpeptidase, and these lower levels were maintained until day 68. Thereafter, both markers gradually increased, prompting re-administration of GC-MO (cycle 2). Following cycle 2, both markers rapidly decreased and reached levels comparable to those observed after cycle 1 by day 194. This improvement persisted until day 250 despite discontinuation of gemcitabine *(Fig. 1)*.

Contrast-enhanced abdominal computed tomography scan performed three months after treatment initiation (day 90) demonstrated shrinkage or disappearance of multiple liver metastases *(Fig. 2A)* and reduction of metastatic intra-abdominal lymph nodes *(Fig. 2B)*. These radiological findings, together with the improvement in liver function markers, suggested a possible additive therapeutic effect of GC-MO therapy.

Immunological analysis showed that interferon-γ–producing cells increased approximately 1.6-fold compared with the pretreatment baseline (day 0) at 43 days after dose 2 in cycle 1 (day 68), which was consistent with the radiological response and the improvement in liver function markers *(Fig. 3A)*. In cycle 2, interferon-γ–producing cells increased 2.6-fold on day 145 (7 days after dose 1) compared with the baseline before cycle 2 (day 138) and remained slightly elevated on day 173, accompanied by a decrease in liver function markers *(Fig. 3B)*.

Regarding adverse events, only mild transient fever (37.1 °C) occurred within 48 hours after GC-MO administration.

After completion of cycle 3, the patient resumed low-dose chemotherapy. However, progression of the primary lesion was subsequently observed, and the patient died on day 454.

DISCUSSION

In this case, repeated administration of GC-MO was temporally associated with improvement in liver function markers, radiological regression of metastatic lesions, and increased systemic immune activation, as indicated by elevated numbers of IFN-γ–producing cells. These findings suggest that iNKT cell–targeted immunotherapy using GC-MO may have contributed to the observed antitumor responses when administered in combination with chemotherapy.

Invariant natural killer T (iNKT) cells are a specialized subset of T lymphocytes that link innate and adaptive immunity. They recognize glycolipid antigens presented by CD1d molecules on antigen-presenting cells and rapidly produce cytokines such as interferon-γ (IFN-γ)

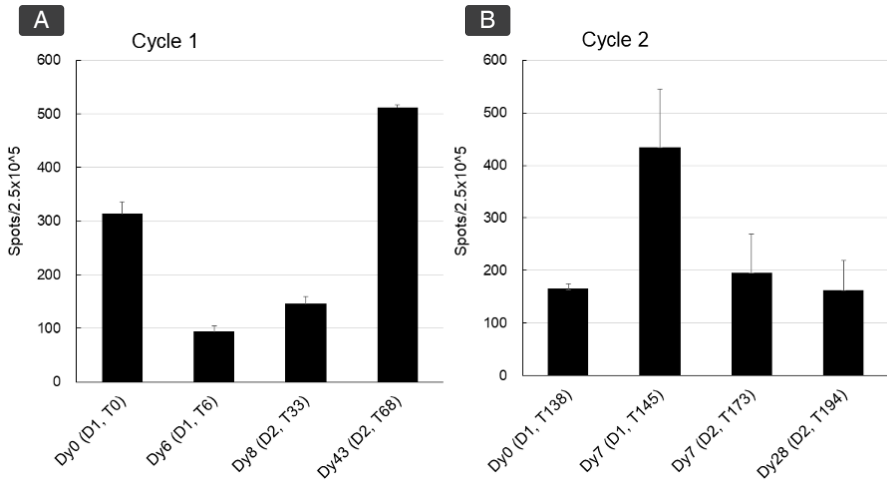


Figure 3. *Interferon-γ–producing cells measured by enzyme-linked immunospot (ELISPOT) assay.*
A) Cycle 1; B) Cycle 2. Peripheral blood mononuclear cells collected during treatment were analyzed using an ELISPOT assay to quantify interferon-γ–producing cells. The x-axis shows Dy (days after each dose), with T (days since treatment initiation) in parentheses. D1 and D2 indicate dose 1 and dose 2, respectively. Data are presented as mean ± standard deviation (n = 3).

and interleukin-4[5]. Through this rapid cytokine release, iNKT cells activate multiple immune effector populations, including natural killer (NK) cells and cytotoxic $CD8^+$ T lymphocytes, thereby promoting antitumor immune responses[5,6].

Several clinical studies have explored the therapeutic potential of iNKT cell–based immunotherapy in advanced malignancies. Administration of α-GalCer–pulsed antigen-presenting cells has been shown to expand and activate iNKT cells in patients with solid tumours and to induce systemic immune responses, including increased IFN-γ production and NK-cell activation[7,8]. In addition, adoptive transfer of ex vivo expanded iNKT cells has been reported to be feasible and safe in patients with advanced cancers and may induce antitumor immune responses[9].

Most previous clinical studies have used α-GalCer–pulsed dendritic cells as antigen-presenting cells. In contrast, the present case utilized $CD14^+$ monocytes (CD14MO) as the antigen-presenting platform. CD14MO are capable of presenting glycolipid antigens via CD1d molecules and activating iNKT cells through this interaction[6,10]. Compared with dendritic cell–based approaches, CD14MO may offer a simpler and potentially more scalable platform for antigen presentation and iNKT cell activation. However, clinical data using CD14MO in iNKT-targeted immunotherapy remain limited.

In the present case, the increase in IFN-γ–producing cells observed after GC-MO administration was temporally associated with both radiological tumour regression and improvement in liver function markers. The repeated administration of GC-MO was also followed by renewed improvement in liver function markers, suggesting that activation of the iNKT–NK cell axis may have contributed to disease control in this patient. In addition to NK-cell activation, iNKT cells can promote dendritic cell maturation and enhance cross-presentation of tumour antigens, thereby facilitating the activation and expansion of tumour-specific $CD8^+$ T cells[6,10]. Activated iNKT cells can also promote NK-cell activation through IFN-γ–dependent signalling and immune-cell crosstalk, thereby amplifying innate antitumor immunity[11].

Reports describing iNKT cell–targeted immunotherapy in GBC are extremely limited. GBC is an aggressive malignancy with a poor prognosis in advanced stages, and effective treatment options remain limited once the disease becomes unresectable. Therefore, strategies that enhance antitumor immunity may be a potentially valuable complementary therapeutic approach.

In this case, gemcitabine chemotherapy was administered concomitantly and may also have contributed to the clinical course. Gemcitabine has been reported to exert immunomodulatory effects, including the reduction of immunosuppressive cell populations such as myeloid-derived suppressor cells, which may enhance antitumor immune responses and potentially improve the efficacy of immunotherapy[12]. Such interactions between chemotherapy and immune activation may partly explain the clinical and immunological responses observed in this patient.

However, several limitations should be considered. First, this report describes a single case, and therefore a causal relationship between immune activation and tumour regression cannot be established. Second, multiple therapeutic interventions, including chemotherapy and Kampo medicine (Juzentaihoto), were administered concurrently, making it difficult to determine the individual contribution of each treatment modality.

Despite these limitations, the temporal association between GC-MO administration, immune activation, and clinical improvement suggests that activation of iNKT cells may have contributed to the observed antitumor effects. Further clinical studies are needed to clarify the interactions among CD14MO, iNKT cells, NK cells, and $CD8^+$ T cells, and to evaluate the potential role of this therapeutic strategy in hepatobiliary malignancies.

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