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NKT Cell-Based Immunotherapy for Non-Small-Cell Lung Cancer

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Abstract

Invariant natural killer T (iNKT) cells are a unique lymphocyte subpopulation that possesses an invariant T cell receptor (TCR) and recognize glycolipid antigens, such as α-galactosylceramide (αGalCer), presenting on CD1d. Activated iNKT cells show a direct and indirect antitumor effect by producing effector molecules and cytokines that activate other immune cells, including NK cells and cytotoxic T cells. We are currently focusing on the development of immunotherapy targeting iNKT cells and have conducted early-phase clinical trials for non-small cell lung cancer (NSCLC). Previous clinical studies have shown that the intravenous injection of α-GalCer-pulsed dendritic cells (DCs) induced the activation of endogenous iNKT cells and iNKT cell-dependent responses. Furthermore, an increase in the number of IFN-γ-producing cells among peripheral blood mononuclear cells has been shown to be associated with prolonged survival. A dramatic infiltration of iNKT cells in the tumor microenvironment was also observed after the injection of αGalCer-pulsed DCs. Based on these results, Phase II clinical trials of αGalCer-pulsed DCs for NSCLC were designed as Advanced Medical Technology and approved by the Japanese Ministry of Health, Labor, and Welfare. Patients with advanced or recurrent NSCLC who had received first-line chemotherapy underwent intravenous injections of αGalCer-pulsed DCs. αGalCer-pulsed DCs were found to be well-tolerated and prolonged overall survival. We also discuss future potential combination therapies of iNKT cell-based immunotherapy to achieve enhanced antitumor activity and provide better treatment options for patients with advanced NSCLC.

Keywords

NKT cells
Immunotherapy
Non-small cell lung cancer
Dendritic cells

1. Introduction

CD1d-bound invariant natural killer T (iNKT) cells express a single T cell receptor (Vα24-Jα18 and Vβ11 chains in humans, and Vα14-Jα18 and Vβ8.2 chains in mice), recognize and activate glycolipid antigens presented on CD1d, an MHC class I-like molecule. Activated iNKT cells exhibit direct cytotoxic activity via the production of cytotoxic molecules such as perforin, and also produce large amounts of cytokines such as interferon-γ (IFN-γ) and inducing the activation of NK cells and $CD8^+$ T cells, thereby exerting a strong antitumor effect [1].

Human iNKT cells are present in the peripheral blood at a very low rate (0.01%–0.1%), making functional analysis difficult. Alpha-galactosylceramide (αGalCer), a sphingolipid, was discovered as a ligand that activates iNKT cells in 1997, and research has developed on the use of the antitumor effects of iNKT cells in cancer therapy [2]. This paper describes the results of clinical trials using αGalCer-pulsed dendritic cells (DCs) for lung cancer as part of the research into the development of immunotherapy targeting iNKT cells and outlines a Phase II study using αGalCer-pulsed DCs conducted as Advanced Medicine B.

2. NKT cell immunotherapy for lung cancer

Lung cancer was the most commonly diagnosed cancer worldwide in 2018, accounting for first place (18.4%) in cancer deaths [3]. In Japan, lung cancer ranked first (25%) among men and second (14%) among women in the projected number of cancer deaths in 2018, and also ranked high in the projected number of cancer cases [4]. In terms of age, lung cancer accounts for a larger proportion of all cancer deaths in people aged 70 years and over. In general, lung cancer is considered to have a poor prognosis, with the overall five-year survival rate for lung cancer being around 40%.

Lung cancer is divided into two main histological types, of which non-small cell lung cancer (adenocarcinoma, squamous cell carcinoma, large cell carcinoma, etc.) accounts for the majority. In particular, chemotherapy has been used as standard treatment for unresectable advanced non-small cell lung cancer (NSCLC), but the radical cure is difficult and the prognosis is extremely poor. In recent years, immunotherapy using immune checkpoint inhibitors has been increasingly indicated, but the efficacy of single-agent chemotherapy is still limited, and there is a need for new therapies that can provide further therapeutic efficacy. In this context, our laboratory aims to establish a treatment targeting iNKT cells, which are involved in a wide range of immune responses, and through several clinical studies in NSCLC and pilot studies using patient samples, we have clarified the efficacy and safety of αGalCer-pulsed DCs, including their ability to prolong overall survival.

3. Establishment of αGalCer-pulsed dendritic cells

Activation of iNKT cells *in vivo* requires a large number of antigen-presenting cells, mainly dendritic cells. However, the conventional method of inducing dendritic cells by culturing $CD14^+$ monocytes in the peripheral blood in the presence of interleukin-4 (IL-4) and granulocyte-macrophage colony-stimulating factor (GM-CSF) does not provide a sufficient number of cells, so an alternative method was sought. Therefore, a new method was found to induce antigen-presenting cell differentiation by culturing whole peripheral blood mononuclear cells from lung cancer patients in the presence of IL-2 and GM-CSF (**Figure 1**) [5]. This method includes not only dendritic cells but also other immune cells, which promotes the maturation of DCs, especially by T cells in the culture system, and enhances the activation and proliferation of iNKT cells compared to conventional methods. All cells, including dendritic cells induced to differentiate by this method, are hereafter referred to as αGalCer-pulsed DCs.

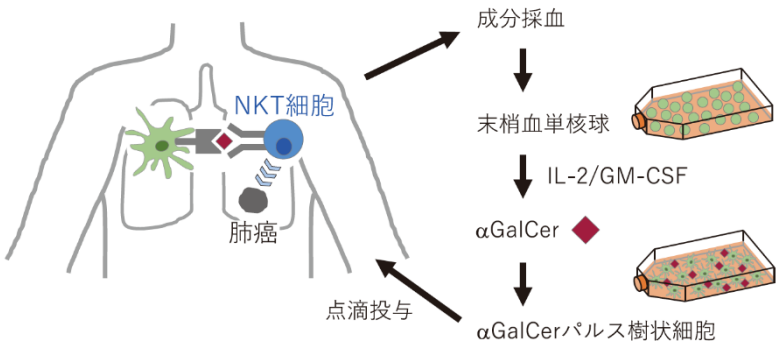

Figure 1. Overview of immunotherapy targeting NKT cells. Peripheral blood mononuclear cells are isolated from lung cancer patients by component blood sampling and cultured in the presence of IL-2 and GM-CSF for 6 or 13 days. One day before administration to the patient, αGalCer is added and all cultured cells including dendritic cells (αGalCer-pulsed dendritic cells) are administered to the patient by intravenous infusion. The intravenously administered αGalCer-pulsed dendritic cells reach the lungs hematogenously and activate NKT cells locally in the tumor.

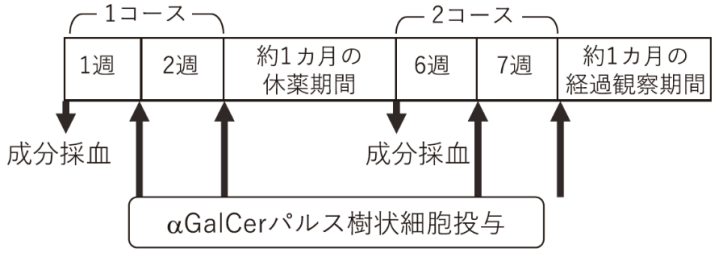

Figure 2. αGalCer-pulsed dendritic cell treatment protocol. One course consists of blood component sampling followed by αGalCer-pulsed dendritic cell administration one and two weeks later, followed by a second course after a one-month withdrawal period.

4. Clinical trials conducted to date: administration of αGalCer-pulsed dendritic cells

4.1. αGalCer-pulsed dendritic cells for advanced unresectable lung cancer: a phase I clinical study

A phase I clinical study was conducted using cells cultured by the method aforementioned [6]. In this clinical study, safety was investigated in subjects with unresectable or post-operative recurrent non-small cell lung cancer who had completed standard treatment, using a dose-escalation method with three conditions (5×10^7 cells/m^2, 2.5×10^8 cells/m^2, and 1×10^9 cells/m^2) for the number of αGalCer-pulsed dendritic cells administered. The protocol treatment was completed in nine of the 11 patients enrolled in the study.

As a result, although no clear tumor-reducing effect was observed, an increase in iNKT cells in the peripheral blood and long-term survival of more than one year was observed in three patients who received 1×10^9 cells/m^2 of αGalCer-pulsed dendritic cells. No grade 2 or higher adverse events were observed in all patients, suggesting the safety of αGalCer-pulsed dendritic cell administration.

4.2. αGalCer-pulsed dendritic cells in advanced unresectable lung cancer: a phase I/II study

Next, a phase I/II clinical trial was conducted in patients with unresectable or postoperative recurrent non-small cell lung cancer at clinical stages IIIB and IV to verify clinical efficacy in addition to safety [7]. Based on the results of the aforementioned phase I clinical study, 1×10^9 cells/m^2 of αGalCer-pulsed dendritic cells were administered by intravenous infusion for a total of four times in two courses. Patients were enrolled after a minimum 4-week withdrawal period following anticancer chemotherapy. Of the 23 patients enrolled, 17 completed the study.

The median survival of the 17 patients was 18.6 months and the 2-year survival rate was 41.2%. According to existing reports, the median survival in large phase III trials of second-line docetaxel-based therapy ranged from 7.0 to 7.5 months [8], whereas in randomized controlled trials of immunotherapy with anti-PD-1 antibodies phase II/III trials ranged from 10.4 to 12.7 months [9]. Although our study was

a phase I/II trial and therefore cannot be compared with other reports, the results of this study were considered sufficient to plan the next study with the primary endpoint of prolonged survival with NKT cell immunotherapy.

Concerning the immune response, αGalCer stimulation increased IFN-γ-producing cells in the peripheral blood more than twofold in 10 patients (good responder group), while a slight increase or no change was observed in the remaining seven patients (poor responder group). The median survival and 2-year overall survival of the two groups were significantly different, with the poor responder group showing a median survival of only 9.7 months and 14.3%, whereas the good responder group showed a very good value of 31.9 months and 60.0%. In the past, it has been shown that the IFN-γ-producing cells shown here are NKT cells and NK cells activated *in vivo* by αGalCer stimulation [10,11]. These results suggest that an increase in the number of IFN-γ-producing cells is associated with prolonged overall survival.

With regard to adverse events, one patient had recurrent grade 3 deep vein thrombosis and was treated with continuous heparin after admission. This patient had developed deep vein thrombosis (DVT) in the left thigh prior to study enrolment and was prescribed warfarin. It was determined by the Effectiveness and Safety Assessment Committee that the recurrent DVT occurred due to an insufficient dose of warfarin and there was no causal relationship with the protocol treatment. No grade 2 or higher toxicity or serious adverse effects were observed in all other patients, indicating the safety of αGalCer-pulsed dendritic cell administration.

4.3. αGalCer-pulsed dendritic cells in resectable advanced lung cancer

In order to clarify the mechanism of clinical efficacy, such as prolongation of overall survival in non-small cell lung cancer patients, as demonstrated in the aforementioned clinical study, it was decided to investigate the effect of αGalCer-pulsed dendritic cells on the tumor microenvironment [12]. In this study, 1×10^9 cells/m^2 of αGalCer-pulsed dendritic cells were administered intravenously seven days before surgery to patients with advanced non-small cell lung cancer at clinical stage IIB or IIIA. To investigate the immune response mechanisms of immune cells, mainly iNKT cells, after αGalCer-pulsed dendritic cell administration, we collected resected tumor tissue, non-tumor lung tissue, lymph nodes, and peripheral blood for immunological analysis. Resected specimens of advanced non-small cell lung cancer that had not been treated with αGalCer-pulsed dendritic cells were used as a control group.

Results showed that a higher proportion of iNKT cells were present in tumor-infiltrating lymphocytes in the αGalCer-pulsed dendritic cells-treated group than in mononuclear cells in normal lung tissue and lymph nodes (0.002%–0.031% vs 0.15%–1.86%). The mean comparison of the proportion of iNKT cells among tumor-infiltrating lymphocytes showed an increasing trend in the αGalCer-pulsed dendritic cell-treated group compared to the control group (0.165% vs 0.68%). Furthermore, when the number of IFN-γ-producing cells in response to αGalCer stimulation was examined, a marked increase in the number of IFN-γ-producing cells in tumor-infiltrating lymphocytes was observed in the αGalCer-pulsed dendritic cells-treated group. These results indicate that the administration of αGalCer-pulsed dendritic cells promotes the local accumulation of iNKT cells in the tumor microenvironment and induces anti-tumour immune responses such as the production of IFN-γ.

5. αGalCer-pulsed dendritic cells for advanced unresectable lung cancer: advanced medical treatment B

In the clinical studies conducted to date, the safety and clinical efficacy of αGalCer-pulsed dendritic

cells administration have been clarified, and a phase II clinical study was conducted as advanced medical treatment B to establish a novel secondary treatment for unresectable advanced stage or recurrent non-small cell lung cancer as NKT cell immunotherapy, and it was reported at the 74th Advanced Medical Technology Review Committee of the Ministry of Health, Labour and Welfare and the 67th Conference on Advanced Medical Care. Patients diagnosed with clinical stage IIIB, IV, or postoperative recurrent non-small cell lung cancer and who had received primary treatment with anticancer drugs were enrolled in the study after a 4-week withdrawal period from the last chemotherapy treatment. The protocol treatment consisted of two courses of αGalCer-pulsed dendritic cells administered intravenously for a total of four times. Based on previous results, the median overall survival expected in this study was 17 months, with a threshold of 8 months, and the required number of patients was designed to be 35. This advanced study, which started in 2012, completed the enrolment of the expected number of 35 patients in 2015, and the follow-up period for all patients was completed in 2017. As a result, the median survival time for all 35 enrolled patients was 21.9 months and the 2-year survival rate was 37.1%. Regarding the number of IFN-γ-producing cells associated with overall survival, as reported previously, the median survival of the good responder group (24 patients) and the poor responder group (8 patients) was 24.7 and 15.1 months, respectively. A trend toward a better prognosis was observed in the good responder group (Logrank test, $P = 0.06$).

Concerning adverse events occurring during the protocol treatment period, one patient developed tumor pain associated with worsening of the current disease and was admitted to the hospital for palliative treatment, which was judged to be serious, but the pain resolved with palliative treatment and was judged not to be causally related to the treatment. No serious adverse events were observed in the other patients.

6. Future issues

A phase II clinical study of αGalCer-pulsed dendritic cells conducted as advanced medical treatment B suggested results comparable to those in previous clinical studies. As a future validation study, a randomized controlled trial with a control group should be conducted. The study design should also be considered for possible treatment in combination with existing standard therapies of anticancer drugs, radiation, and surgery, for example, durvalumab in chemotherapy combined with radiotherapy plus maintenance for clinical stage IIIB patients [13], pembrolizumab in combination with platinum-based chemotherapy for stage IV patients [14,15], and preoperative nivolumab in resectable advanced lung cancer [16]. Furthermore, we are also examining the possibility of combination therapy with immune checkpoint inhibitors and NKT cell immunotherapy, given that immune checkpoint inhibitors have become the standard of care for primary and secondary treatment of non-small cell lung cancer [17]. With the advent and application of immune checkpoint inhibitors, the current practice guidelines for non-small cell lung cancer treatment require frequent revision, and new treatment options are being reported day by day. Until a cure for advanced lung cancer is achieved, efforts to improve the standard of care will continue, and we would like to contribute to improving the outcome of non-small cell lung cancer by clarifying the role of NKT cell-based immunotherapy in this context through clinical trials.

Disclosure statement

The authors declare no conflict of interest.

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