Increased survival time of a patient with metastatic malignant melanoma following immunotherapy: A case report and literature review

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Abstract. Metastatic malignant melanoma is treated with chemotherapy and radiotherapy. A number of previous studies have indicated that cytokine-induced killer cells (CIK cells) are a heterogeneous cell population that express cluster of differentiation (CD)3 and CD56, in addition to the natural killer cell NKG2D activating receptor. CIK cells possess major histocompatibility complex-unrestricted cytotoxicity towards cancer, but not towards normal targets. The present study investigated whether the addition of CIK cells resulted in an improved therapeutic response in a patient with metastatic malignant melanoma. In the current case, a patient with metastatic malignant melanoma received CIK therapy, which resulted in a relatively long survival time of 28 months. To the best of our knowledge, there have been no previous studies reporting such positive effects in a patient who received CIK cell immunotherapy. Based on the findings of the present study, CIK cell therapy may be an option that results in a good prognosis in certain patients with metastatic malignant melanoma.

Introduction

Research indicates that the annual incidence of melanoma continues to increase (1,2). Metastatic melanoma has a poor prognosis with a median survival time of 6-8 months and a 5-year survival rate of ~6% with chemotherapy (3,4).

At present, immunotherapy is the fourth most common treatment approach for solid tumors, following surgery, chemotherapy and radiotherapy (5,6). Numerous immunotherapy approaches using various killer cells for solid tumors have been reported in the literature, including tumor-infiltrating lymphocytes (TILs), lymphokine-activated killer cells (LAKs) and anti-CD3 monoclonal antibody-induced killer cells (7-9). The anti-tumor functions of TILs are major histocompatibility complex (MHC)-restricted and a number of patients with solid tumors are not eligible for TIL-based therapy since their TILs do not expand sufficiently, their tumors have lost expression of antigens or MHC molecules or they possess extremely low numbers of TILs (7). In addition, LAK and anti-cluster of differentiation (CD)3 monoclonal antibody-induced killer cells exhibit low anti-tumor activities (10). Therefore, due to their limited therapeutic efficacy, they are rarely used in the clinic. In 1991, Schmidt-Wolf *et al* (11) observed a novel type of antitumor effector cell, which was termed a cytokine-induced killer (CIK) cell. CIK cells proliferate rapidly *in vitro*, and possess strong antitumor activity against a broad spectrum of solid tumors (12). A number of clinical trials have reported that treating patients with metastatic solid tumors using CIK cells, alone or in combination with chemotherapy, significantly improves the median survival time, and in addition, that CIK cell transfusions may improve the immune function of patients (12-14). There is limited data on the use of CIK cell therapy for metastatic malignant melanoma (15). In the present study, a patient with metastatic malignant melanoma received CIK cell immunotherapy, which resulted in a relatively long overall survival time (OS) of 28 months

Case report

On November 1st, 2010, A 47-year-old female was admitted to The Affiliated Cancer Hospital of Zhengzhou University (Zhengzhou, Henan, China) with multiple skin metastatic melanoma nodes in the left leg (Fig. 1). Considering that metastatic malignant melanoma is resistant to chemotherapy and radiotherapy, CIK cell immunotherapy was administered. Mononuclear cells were collected from 50 ml of the patient's peripheral blood and cultured in GT-T551 medium (Takara, Tokyo, Japan) containing anti-CD3 antibody (mouse-anti human monoclonal IgG_{2a}; catalog no. sc-19590; Santa Cruz Biotechnology, Inc., Dallas, TX, USA; dilution, 1:300),

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Table I. Published CIK cell clinical studies.

Year	Tumor type	No. of cases	Culture condition	Therapeutic approach	Clinical response
1999 (16)	RCC, CC, lymphoma	10	IFN-γ, anti-CD3, IL2 plus IL-2 transgene	Auto-CIKs transfected with IL-2 gene	1 CR, 3 SD, 6 PD
2006 (17)	GC	57	IFN-γ, anti-CD3, IL2 and IL-1α	Arm 1: Chemotherapy Arm 2: Auto-CIKs combined with chemotherapy	Arm 1: 4 PR, 8 MR, 6 SD, 7 PD Arm 2: 7 PR, 11 MR, 8 SD, 6 PD
2008 (18)	NSCLC	59	IFN-γ, anti-CD3, IL2 and IL-1α	Arm 1: Chemotherapy Arm 2: Auto-CIKs combined with chemotherapy	Arm 1: ORR, 43.3%; TTP, 4.67; OS, 11 Arm 2: ORR, 44.8%; TTP, 6.65; OS, 15
2009 (19)	HCC	127	IFN-γ, anti-CD3, IL2 and IL-1α	CIK-I group: 3 courses CIK-II group: 6 courses Control group: none	The 1-year and 18-month recurrence rates of the CIK-I and CIK-II groups were lower than that of the control group
2012 (14)	RCC	148	IFN-γ, anti-CD3, IL2 IL-1β	Arm 1: Auto-CIKs Arm 2: IL-2 combined with IFN-α	Arm 1: 13 CR, 26 PR, 25 SD, 10 PD Arm 2: 5 CR, 15 PR, 25 SD, 29 PD
2013 (20)	PC	1	IFN-γ, anti-CD3, IL2 and IL-1α	Auto-CIKs	CR, PFS ≥19 months.
2013 (21)	HCC	174	IFN-γ, anti-CD3, IL2 and IL-1α	Arm 1: Auto-CIKs+TACE+RFA; Arm 2: TACE+RFA.	Arm 1: PFS, 17; OS, 56 Arm 2: PFS, 10; OS, 31

CIK, cytokine-induced killer; RCC, renal cell carcinoma; CC, colorectal cancer; GC, gastic cancer; NSCLC, non-small cell lung cancer; HCC, hepatocellular carcinoma; PC, prostate cancer; IFN, interferon; CD, cluster of differentiation; IL, interleukin; TACE, transcatheter arterial chemoembolization; RFA, radiofrequency ablation; CR, complete response; SD, stable disease; PD, progressive disease; PR, partial response; MR, minimal response; ORR, overall response rate; TTP, time to progression; OS, overall survival; PFS, progression-free survival.

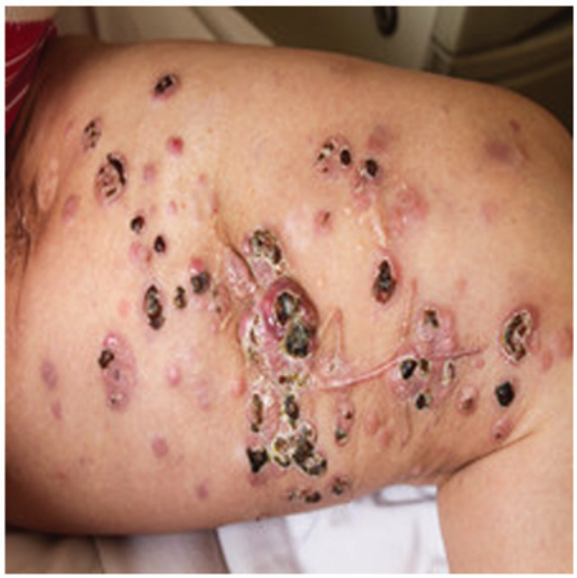

Figure 1. Metastatic melanoma nodes in the patient's left leg prior to cytokine-induced killer cell therapy.

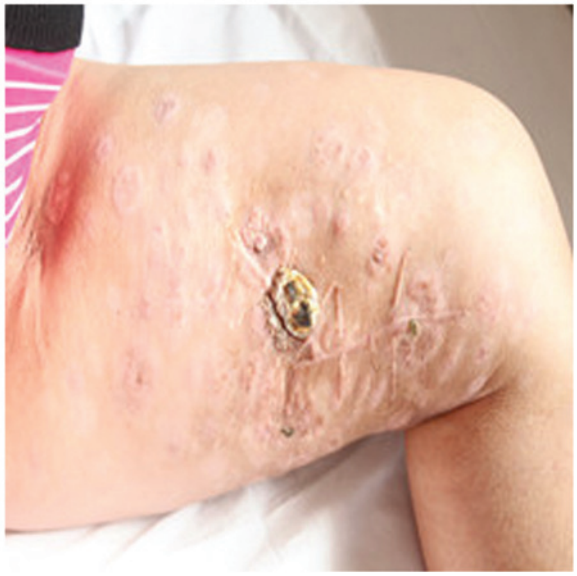

Figure 2. Metastatic melanoma nodes in the patient's left leg following cytokine-induced killer cell therapy.

recombinant human interleukin-1α (IL-1α; 1,000 U/ml) and interferon-γ (IFN-γ; 1,000 U/ml), at 37˚C with 5% CO_2 for 24 h. Next, recombinant human IL-2 (1,000 U/ml) was added to the medium. The medium was replaced with fresh IL-2 and IFN-γ-containing medium every 5 days. At day 10, the CIK cells were harvested and their phenotype was analyzed. All products were free of bacterial, mycoplasma and fungal contamination. The endotoxin activity was ≤5 EU. Phenotypic analysis of the autologous CIK cells in the patient prior to culture and following 10 days of culture demonstrated that the expression of certain antigens in the CIK cells increased during this time as follows: $CD3^+$, from 44.39±6.21 to 91.36±8.72%; $CD3^+/CD4^+$, from 30.08±4.78 to 45.63±7.79%; $CD3^+/CD8^+$, from 17.96±6.35 to 36.80±4.45%; $CD3^+/CD56^+$, from 3.68±1.47 to 11.98±3.91%; and $CD25^+$, from 17.23±4.45 to 32.67±5.79%, respectively, with $P<0.05$ for all differences (*t*-test; SPSS software 11.0; SPSS, Inc., Chicago, IL, USA). The results indicated that the percentages of $CD3^+$, $CD3^+/CD4^+$, $CD3^+/CD8^+$ and $CD25^+$ cells were significantly increased following stimulation and expansion in culture, which is crucial to tumor immunity. The total number of CIK cells transplanted in 1 cycle of CIK therapy was ~$5x10^9$ cells. Between 10 November 2010 and 27 June 2011, the patient received 8 cycles of CIK cell immunotherapy and $2x10^6$ units of IL-2 at days 1-5 per CIK cell infusion. No adverse reactions were observed during the period of CIK cell therapy. Following 8 cycles of CIK cell immunotherapy, the size and number of metastatic melanoma nodes in the patient's left leg were notably reduced (Fig. 2). The patient underwent re-examination every 3 months. At 1 year after the patient's first CIK cell treatment, re-examination indicated a stable disease state. The patient then received 4 additional cycles of CIK therapy. However, 2 years later, an abdominal contrast-enhanced CT scan indicated that multiple nodes were present in the patient's liver and the treatment was discontinued. The patient succumbed to the disease on March 10, 2013. The overall survival time was 28 months.

Written informed consent was obtained from the patient's family for the publication of this study.

Discussion

Patients with metastatic melanoma have a median survival of 6-8 months and a 5-year survival of 6% (3,4). FDA-approved treatments for metastatic melanoma including IL-2, chemotherapy, ipilimumab, vemurafenib, nivolumab and so on, and the median survival time given these treatments is from 6-24 months (15,22,23). Although the survival time of metastatic melanoma patients has improved, novel regimens remain critical to increase it further. In 1991, Schmidt-Wolf *et al* (11) first reported that CIK cells possessed potential antitumor cell activity in the immunodeficient SCID mouse/human lymphoma model. Following this finding, additional studies indicated that CIK cells may be a better choice for patients with solid tumors and demonstrated that CIKs possess strong antitumor activity *in vitro* (24-26). A number of previous studies have also demonstrated that CIKs may reverse drug resistance in solid tumor cells (24-26). Therefore CIK cell therapy has been proposed as an alternative therapeutic strategy to adopt for patients with solid tumors. Previous studies have demonstrated that CIK cells are a novel and heterogeneous population of immune effector cells with a high proliferation rate and potent cytotoxic activity against a variety of solid tumors, including renal cell carcinoma, lung cancer and prostate cancer, alone or together with chemotherapy compared with conventional therapies (Table I) (12,14,20).

Since CIK cells possess strong antitumor activity and high amplification efficiency, increasing numbers of studies have administered CIK cell therapy alone or in combination with chemotherapy. Previous studies have demonstrated that CIK cells are a heterogeneous cell population, which express CD3 and CD56 in addition to the natural killer cell NKG2D

activating receptor. CIK cells possess MHC-unrestricted cytotoxicity towards solid tumors, but not toward normal targets, and may regulate and enhance the cellular immune functions in patients with solid tumors by the secretion of cytokines, such as interferon-γ, and a number of chemokines, including RANTES, MIP-1α and MIP-1β (27-30). However, there is currently a lack of studies in which CIK cells were used to treat metastatic malignant melanoma in clinical practice (31). The current case study presents a novel choice of treatment, however, additional cases are required to verify its efficacy.

In conclusion, the results of the present study demonstrate that CIK cells may be easily expanded *in vitro*, and their transplantation may result in a significant treatment effect in patients with metastatic malignant melanoma. Furthermore, CIK cell therapy is a safe technique that exhibits potential efficacy against metastatic malignant melanoma by significantly enhancing immune function, by increasing the absolute effector cell number without notable side-effects. These results may have a favorable impact on conventional treatments if applied in large-scale studies.

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