Abstract. Metastatic renal cell carcinoma (RCC) has a poor response to anticancer chemotherapy and radiotherapy. While immunotherapy and molecular targeted drugs have been used as first-line therapy for RCC metastasis, the response rate to these agents is low. We report the case of a patient with lung and bone metastases of RCC whose lung metastases disappeared after reconstruction using the resected specimen treated by liquid nitrogen for the bone metastasis. This 60-year-old female had a left RCC with multiple lung metastases and a left femoral bone metastasis at the time of diagnosis. After left nephrectomy followed by immunotherapy, we performed tumour excision and reconstruction with frozen recycled autograft. The lung metastases had disappeared by 10 months after surgery, while serum levels of interferon-gamma and interleukin-12 had increased. We postulate that the antitumour activity resulted from immunotherapy plus cryotreatment of her bone metastasis and believe that this case supports continued research into immunotherapy for cancer.

Surgery followed by reconstruction using an autograft containing tumour treated by liquid nitrogen is a method of treating malignant bone tumours that we have been employing at our institution. The operative procedure is as follows: after en bloc excision of the tumour, the specimen is immediately frozen in liquid nitrogen for 20 minutes to kill cancer cells, followed by gradual thawing and reimplantation with suitable internal fixation (1-3). Hypothermia not only induces tumour cell death but may also activate a systemic antitumour immune response stimulated by tumour antigens released by the cryonecrotic tissue (4). Renal cell carcinoma (RCC) is increasing in incidence worldwide by approximately 2% per year (5). For the approximately 25-30% of RCC patients who have metastatic disease (mRCC) at diagnosis, prognosis is extremely poor, with a typical 5-year survival rate less than 11% (6). Although cytokine immunotherapy has been in use for several decades, evidence to date suggests that only a small proportion of patients with mRCC derive clinical benefit from immunotherapy with interleukin-2 (IL-2) or interferon-alpha (IFN-α) (5). We report herein on a patient with lung and bone metastases of RCC whose lung metastases disappeared after reconstruction using the resected specimen treated by liquid nitrogen for bone metastasis in conjunction with IL-2 and IFN-α immunotherapy.

Case Report

A 60-year-old woman presented to our hospital with left knee pain. Radiography demonstrated osteolytic changes in the left distal femur. Fluorodeoxyglucose positron-emission tomography (FDG-PET) scan showed accumulation in the left kidney and left distal femur, and computed tomography revealed multiple lung nodules. We diagnosed left RCC with concurrent bone and lung metastases (pT3N0M1). The patient underwent radical nephrectomy for RCC of her left kidney, and the histological findings showed clear cell carcinoma. Two weeks later, she began receiving immunotherapy with intramuscular natural IFN-α (Sumiferon) at a dosage of 6 million units and IL-2 (Teceleukin) at a dosage of 700000 units five times per week. Subsequent radiograms and computed tomography (CT) studies demonstrated that the number and size of her lung metastases had not increased a month later, but her bone metastasis had doubled in size during that time, such that CT showed a large, low-density 3×3 cm mass in the medial part of the distal femur associated with a thin to absent bony cortex.

We performed cryotreatment of this bone metastasis consisting of wide excision and reconstruction using the

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The resected specimen which had been treated by liquid nitrogen. The surgical procedure began with a proximal femoral osteotomy with a 3-cm-wide margin from the tumour. Soft tissue was divided along the extremity beyond a safe surgical margin until the arc of rotation was adequate. Next, the bony lesions connecting with the limb were rotated cautiously, frozen in liquid nitrogen for 20 minutes, and then thawed at room temperature for 15 minutes followed by further thawing in distilled water for an additional 10 minutes. Reconstruction after freezing was performed using antibiotic-loaded cementation with a plate attached by screws (Figure 1 and 2). Some tumour tissue invading into the bony trabecular was probably left in the lesion.

Two weeks after the operation, we initiated immunotherapy with intramuscular natural IFN-α (Sumiferon) at a dose of 6 million units five times a week for 28 months. We studied the associated immune reactions by collecting blood samples from the patient before surgery and one month after surgery to measure the levels of interferon-γ (IFN-γ, human, ELISA Kit; Quantikine R and D Systems, Minneapolis, MN, USA) and interleukin-12 (IL-12, human, ELISA Kit; Quantikine R and D Systems) cytokines. The preoperative IFN-γ level was 12.0 (IU/ml), increasing to 33.7 (IU/ml) one month after surgery. Similarly the level of IL-12 increased from a preoperative value of 12.0 (pg/ml) to 79.7 (pg/ml) one month after surgery. Meanwhile, the patient’s lung metastases showed decreases in their size during this first month and had disappeared by 10 months after the operation (Figure 3). Thirty months after the operation, there is still no sign of the lung metastasis, and the patient has no evidence of disease.

Discussion
This case study analyzed a patient with mRCC with lung and bone metastases whose lung metastases disappeared after excision of the bone metastasis and reconstruction using the resected specimen treated by liquid nitrogen in conjunction with immunotherapy. Cryotreatment of a bone tumour using a frozen specimen treated by liquid nitrogen is a type of cryosurgery, and previous reports have suggested immune system activation by cryosurgery. Ablin et al. described several patients in whom spontaneous regression of metastases occurred after cryosurgery of a primary prostate tumour (7). In animal models, cryosurgery of tumour tissue resulted in rejection of a tumour re-challenge (8) and inhibition of secondary and metastatic tumour growth (9). TNF-α, IFN-γ and IL-12 levels have shown increases in several cryosurgery models (4, 8, 9). Increases in cytotoxic activity and natural killer T-cell activity have been measured in lymphocytes from lymph nodes or spleens that are recipient sites of tumour drainage (8). These reports indicate that cryosurgery not only destroyed tumour tissue directly by freezing and thawing but also induced a specific antitumour effect by an immune mechanism that was stimulated by tumour antigens released by cryonecrotic tissue (4). We reported that re-implantation of tumour tissue treated by cryotreatment with liquid nitrogen induces antitumour activity against murine osteosarcoma (10). We also reported that the serum levels of IFN-γ and IL-12 increased after cryotreatment with liquid nitrogen against tumor tissue (11). This antitumour effect enhanced the systemic immune response.
reaction and reduced tumour growth and metastasis in the living body. In our patient, we observed increases in the serum levels of IFN-γ and IL-12 after reconstruction using a frozen specimen treated by liquid nitrogen, which suggests that the tumour antigens released by the frozen tumour tissue induced antitumour immunological activation.

The response of mRCC to anticancer chemotherapy or radiotherapy is poor (6). While immunotherapy, such as IFN-α or IL-2, is an effective form of treatment against pulmonary metastases for the RCC patients who respond to these agents, response rates are low, only 10-30% for IFN-α and about 20% for IL-2, for example, which means that
immunotherapy overall has only limited utility (5, 12). Recently, molecular target drugs (sorafenib and sunitinib) have been used as first-line therapy for RCC metastasis or second-line therapy for immunotherapy-resistant RCC metastasis. However, these molecular targeted treatments have not yet demonstrated long-term benefits (13, 14).

Our patient received combination therapy of IL-2 and IFN-α to treat her lung metastases and bone metastasis. This regimen proved to be ineffective for preventing growth of her bone metastasis and only stabilized the size of her lung metastases. After the patient underwent surgery for her bone metastasis and reconstruction using a frozen specimen treated by liquid nitrogen, her lung metastases showed shrinkage at 1 and 3 months and had completely disappeared by 10 months after surgery. In this case, a frozen specimen treated by liquid nitrogen not only reduced bone metastasis but also might have induced a specific antitumour effect that, in combination with the cytokine therapy, appears to have synergistically potentiated the immune reaction. Moreover, additional effect to cryoimmunology, the combination of the treatment with liquid nitrogen and specific tumour antigen-pulsed dendritic cell immunotherapy will be available in basic research (15).

In conclusion, in a patient with mRCC involving the lungs and bone, the patient’s lung metastases disappeared after reconstruction using the resected specimen treated by liquid nitrogen for the bone metastasis in conjunction with cytokine immunotherapy. These findings suggest that in RCC patients with metastases to lung and bone, cryotreatment of the bone metastasis may potentiate the efficacy of immunotherapy and in some cases cause shrinkage or even disappearance of lung metastases.

References