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# Cancer cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer as vaccines for the treatment of genitourinary malignancies

**Abstract** When irradiated and administered intradermally as vaccines, cancer cells engineered to secrete high levels of granulocyte-macrophage colony-stimulating factor (GM-CSF) by gene transfer elicit potent anticancer immune responses in a variety of animal tumor models. Upon vaccination, antigens present in the cancer cells are phagocytosed and processed by skin dendritic cells. These dendritic cells then prime anticancer immune responses by presenting antigenic peptides to T cells. The immune responses generated are capable of eradicating small but lethal cancer cell inocula with minimal toxicity in preclinical animal tumor studies. To develop this vaccination strategy for the treatment of human genitourinary cancers, we have conducted phase I clinical trials using human genitourinary cancer cells as sources of cancer cell antigens. In the first human clinical trial of genetically engineered cancer cell vaccines, a phase I clinical trial of kidney cancer cell vaccines (n = 18), kidney cancer cells were removed at surgery, propagated briefly in vitro, and then genetically modified to secrete high levels of GM-CSF via ex vivo transduction with the retrovirus MFG-GM-CSF. After irradiation, the kidney cancer cells were administered as vaccines to 18 patients with advanced kidney cancers. Vaccine treatment, which caused few side effects, nonetheless appeared to trigger anticancer immune responses manifest as conversion of delayed-type hypersensitivity (DTH) skin responses against irradiated autologous cancer cells after vaccination. Biopsies of vaccine sites yielded findings reminiscent of biopsies from preclinical animal model studies, with evidence of vaccine cell recruitment of dendritic cells, T cells, and eosinophils. One patient with measurable kidney cancer metastases treated at the highest vaccine dose level experienced a partial treatment response. The bioactivity of GM-CSF-secreting autologous cancer cell vaccines was confirmed in a phase I clinical trial for prostate cancer (n = 8). Vaccine cells were prepared from surgically harvested prostate tumors by ex vivo transduction with MFG-GM-CSF in a manner similar to that used for the kidney cancer trial. Vaccine treatment was well tolerated and associated with induction of anticancer immunity as assessed using DTH skin testing. In addition, new antiprostate cancer cell antibodies were detected in serum samples from treated men as a consequence of vaccination. These first clinical trials of GM-CSF-secreting cancer cell vaccines for the treatment of genitourinary cancers have demonstrated both safety and bioactivity, in that very few side effects have been seen and anticancer immune responses have been detected. Future clinical studies will be required to assess vaccine treatment efficacy, refine vaccination dose and schedule, define the appropriate clinical context for the use of such

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vaccines, and ascertain optimal combinations involving vaccines and other local or systemic anticancer treatments.

**Key words** Kidney cancer · Prostate cancer · Granulocyte-macrophage colony-stimulating factor · Vaccine · Gene transfer

## Introduction

Granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting cancer cell vaccines, prepared using gene transfer, have been demonstrated to elicit potent anticancer immune responses in a variety of animal models [5], including animal models of kidney cancer [5, 11] and prostate cancer [2, 12, 16]. Skin dendritic cells (DCs) likely play a critical role in the generation of anticancer immune responses in animals administered intradermal injections of irradiated GM-CSF-secreting cancer cell vaccines (Fig. 1). In response to vaccination, DCs activate antigen-specific $CD4^+$ T cells that orchestrate a portfolio of immune responses, ultimately involving the participation of $CD4^+$ and $CD8^+$ T cells, B cells, macrophages, and eosinophils [6, 7]. Clinical translation of this new immunotherapy approach for the treatment of human kidney cancer and prostate cancer (PCA) requires isolation of cancer cells by surgical harvest, propagation of the cells ex vivo, transfer of cDNA encoding GM-CSF into the cultured cancer cells to create GM-CSF-secreting vaccine cells, irradiation of the vaccine cells to prevent growth and maximize transfer of cancer cell antigens to skin DCs, and inoculation of irradiated GM-CSF-secreting vaccine cells into skin sites in patients with advanced kidney cancer and PCA. Here, we review the results of phase I clinical trials of GM-CSF-secreting cancer cell vaccines for kidney cancer [1, 13] and PCA [14].

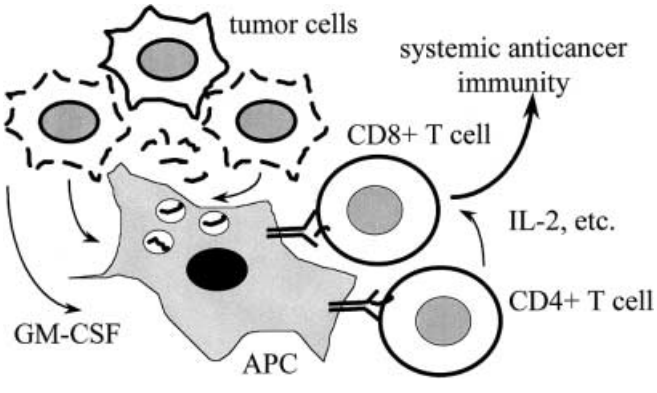


**Fig. 1** GM-CSF-secreting cancer cell vaccines prime anticancer immune responses via transfer of cancer antigens from dying cancer cells to antigen-presenting cells (APCs) at vaccine sites in the skin (dendritic cells [DCs])

## Patients and methods

For the phase I trial of GM-CSF-secreting cancer cell vaccines for kidney cancer, eligible patients had stage III T4b (tumor thrombus in the inferior vena cava above the level of the diaphragm) or stage IV (metastatic) renal cell carcinoma (RCC), good performance status (Eastern Cooperative Oncology Group status 0 or 1), no cancer treatment within one month of study entry, no history of autoimmune disease, no history of immunotherapy or immunosuppression within one month of study entry, no active infections, and adequate bone marrow, liver, and kidney function [1, 13]. The 18 enrolled patients (10 men and 8 women) were between 44 and 79 (median 59) years old, manifested excellent performance status, and were minimally pretreated for kidney cancer. Vaccine cells were prepared from surgical specimens via establishment of cell cultures; RCC cells were transduced with the MFG-GM-CSF (G-VAX®, CellGenesys, Foster City, CA, USA) retrovirus [4, 9], propagated to permit expansion in vaccine cell number, and then assessed for GM-CSF production by enzyme-linked immunosorbent assay (ELISA) [13]. The study design involved treatment with irradiated autologous RCC cells, prepared with or without *MFG-GM-CSF* gene transduction, in a vaccine cell dose-escalation fashion, from $4 \times 10^6$ cells (dose level 1) to $4 \times 10^7$ cells (dose level 2) to $4 \times 10^8$ cells (dose level 3).

RCC cells prepared without *MFG-GM-CSF* gene transduction exhibited GM-CSF production rates of between 1 and 19 ng/$10^6$ cells/24 h; RCC cells transduced with *MFG-GM-CSF* genes exhibited GM-CSF production rates of between 17 and 149 ng/$10^6$ cells/24 h, with seven of eight vaccine preparations secreting more than the 35 ng/$10^6$ cells/24 h level thought to be necessary for induction of anticancer immune responses in preclinical models [5, 10, 13]. After irradiation at 150 Gy, the cancer cell vaccines were inoculated into skin sites every 28 days. Treated patients were monitored for side effects associated with vaccination, for evidence of anticancer immunity using delayed-type hypersensitivity (DTH) testing against unmodified autologous RCC cells, for safety of retroviral gene transfer, and when possible, for kidney cancer treatment responses. Sixteen of 18 treated patients were fully evaluable for study endpoints.

The phase I trial of GM-CSF-secreting cancer cell vaccines for PCA involved patients (men) undergoing curative prostatectomy who were discovered to have metastatic PCA at surgery. Eligible patients had PCA complicated by lymph node metastasis, seminal vesicle invasion, or both, as well as good performance status (Eastern Cooperative Oncology Group status 0 or 1), no recent cancer treatment, no history of autoimmune disease, no recent history of immunotherapy or immunosuppression, no active infections, and adequate bone marrow, liver, and kidney function [14]. Although 11 men were enrolled in the trial, a sufficient quantity of vaccine cells was available for only eight of the men; the eight men treated ranged in age between 46 and 64 (median 51) years old, manifested excellent performance status, had a median preoperative prostate-specific antigen (PSA) level of 28.9 ng/mL (range 6.7 to 75 ng/mL), and a median postoperative PSA level at the time of vaccine administration of 0.65 ng/mL (range 0.1 to 30.4 ng/mL).

As was the case for the RCC vaccine cells, PCA vaccine cells were prepared from surgical specimens via establishment of cancer cell cultures, transduction with the *MFG-GM-CSF* retrovirus, cell propagation to permit expansion in number, and then assessment for GM-CSF production by ELISA. The study design involved treatment with irradiated autologous PCA cells, prepared with *MFG-GM-CSF* gene transduction, in an abbreviated vaccine cell dose-escalation fashion, from $1 \times 10^7$ cells (dose level 1) to $5 \times 10^7$ cells (dose level 2). PCA cells transduced with *MFG-GM-CSF* genes exhibited GM-CSF production rates of between 143 and 1403 ng/$10^6$ cells/24 h (GM-CSF production rates before *MFG-GM-CSF* gene transduction were $< 0.2$ ng/$10^6$ cells/24 h; see reference 14). The genetically modified PCA vaccine cells were irradiated and injected into skin sites every 21 days until the vaccine supply was depleted (three to six inoculations). Treated patients were

monitored for PCA vaccine side effects, for retrovirus gene transfer safety, and for evidence of anticancer immunity using DTH testing against unmodified autologous PCA cells and immunoblot analysis for the detection of antiprostate cancer antibodies.

## Results

In both phase I clinical trials, cancer cell recovery and expansion limited the feasibility of the GM-CSF-secreting autologous cancer cell vaccine approach (Table 1). The specific polypeptide antigens best targeted by vaccine immunotherapy for RCC and PCA have not been defined. By using intact autologous cancer cells isolated from each patient for vaccine cell construction, we had hoped to capture many or all candidate RCC or PCA antigens for immune activation with a minimum of bias. However, the optimized cell culture techniques used for RCC and PCA vaccine cell expansion were barely adequate. For RCC vaccines, cell expansion to specified numbers was successful in 70% of dose level 1 patients, 88% of dose level 2 patients, and 20% of dose level 3 patients [13]. For PCA vaccines, primary PCA cultures were established in only 73% of patients, while among the established PCA cultures, cell expansion to specified numbers was successful in 75% of dose level 1 patients and in 43% of dose level 2 patients [14].

Retroviral transfer of the *MFG-GM-CSF* gene was considerably more successful. For both RCC and PCA vaccines, *MFG-GM-CSF* gene transfer increased production of GM-CSF by vaccine cells to levels ($>35$ ng/$10^6$ cells/24 h) thought to be adequate for induction of anticancer immune responses [5, 10].

In both of the phase I trials, side effects associated with vaccine administration were minimal (Table 2). In the RCC trial, systemic side effects attributable to GM-CSF-secreting RCC cell vaccines were limited to pruritis away from the vaccine site appearing in 43% of treatment cycles at dose level 2 [13]. In the PCA trial, systemic side effects, including low-grade fever, chills, and malaise, were encountered in as many as 4% of dose level 1 vaccine treatment cycles and 37% of dose level 2 vaccine treatment cycles [14]. Essentially all patients vaccinated with GM-CSF-secreting RCC or PCA cell vaccines manifested vaccine site side effects, including erythema, induration, pruritis, and tenderness [13, 14]. Similar cutaneous reactions, although somewhat less intense, were noted in patients receiving RCC vaccines prepared without *MFG-GM-CSF* gene transfer. No replication-competent retrovirus was detected in any of the vaccinated patients at any time in either clinical trial. In addition, none of the patients in either trial exhibited signs or symptoms of autoimmune disease.

**Table 1** GM-CSF-secreting RCC and PCA vaccines (*GM-CSF* granulocyte-macrophage colony-stimulating factor, *RCC* renal cell carcinoma, *PCA* prostate carcinoma)

| Vaccination with | Dose level (no./dose) | GM-CSF secretion (ng/$10^6$/24 h) | Patients treated (no.) |
|---|---|---|---|
| *MFG-GM-CSF*-transduced RCC cells | 1 ($4 \times 10^6$) | 17–99 | 4 |
| | 2 ($4 \times 10^7$) | 42–149 | 5 |
| Nontransduced RCC cells | 1 ($4 \times 10^6$) | 1 | 3 |
| | 2 ($4 \times 10^7$) | 1–19 | 5 |
| | 3 ($4 \times 10^8$) | 6–7 | 5 |
| *MFG-GM-CSF*-transduced PCA cells | 1 ($1 \times 10^7$) | 143–607 | 5 |
| | 2 ($5 \times 10^7$) | 233–1403 | 3 |

**Table 2** GM-CSF-secreting RCC and PCA vaccine side effects (*RCC* renal cell carcinoma, *PCA* prostate carcinoma)

| | RCC vaccine trial | PCA vaccine trial |
|---|---|---|
| Evaluable treatment cycles[a] | 15 (7 patients) | 41 (8 patients) |
| Systemic side effects | | |
| Low-grade fever | 0/15 | 2/41 |
| Chills | 0/15 | 3/41 |
| Malaise | 0/15 | 5/41 |
| Other[b] | 8/15 | 0/41 |
| Vaccine site side effects | | |
| Erythema/swelling | 13/15 | 41/41 |
| Tenderness | 7/15 | 10/41 |
| Pruritis | 3/15 | 13/41 |
| Pain during injection | 15/15 | 41/41 |

[a] Includes only patients receiving *MFG-GM-CSF*-transduced cancer cell vaccines

[b] Nonvaccine site pruritis in 2 cycles, constipation in three cycles, urticaria in two cycles, deep venous thrombosis at nonvaccinated limb in one cycle

Biopsies of vaccine sites in the RCC and PCA clinical trials demonstrated recruitment of immune effector cells to sites of autologous cancer cell antigen deposition (Fig. 2). In patients receiving RCC vaccines, the intensity and character of immune cell infiltration increased with increasing vaccine dose and with *MFG-GM-CSF* gene transfer [13]. At dose level 2, vaccine sites containing irradiated GM-CSF-secreting RCC cells displayed infiltration of macrophages, granulocytes, and eosinophils by three days after vaccine inoculation, with infiltration of T cells and DCs more evident by 7 days after vaccination [13]. In men treated with PCA vaccines, inflammatory infiltrates also appeared more intense at higher vaccine dose levels [14]. At these vaccine sites, DCs, macrophages, T cells, and eosinophils all appeared to be present [14].

To assess induction of anticancer immune responses, DTH testing was attempted in both of the phase I clinical trials. The DTH reaction was measured as bidimensional induration at 48 h at skin sites of deposition of irradiated unmodified autologous cancer cells ($1 \times 10^6$ cells in 0.2 mL of buffered saline solution). In each trial, DTH reactivity was assessed before vaccination and after vaccine administration. Control DTH testing was conducted against seven common recall antigens using the Multitest CMI (Connaught Laboratories, Swiftwater, PA, USA). In the RCC trial, although minimally reactive DTH tests against unpassaged RCC cells were present in two patients, significant increases in DTH reactivity were evident in all patients treated at

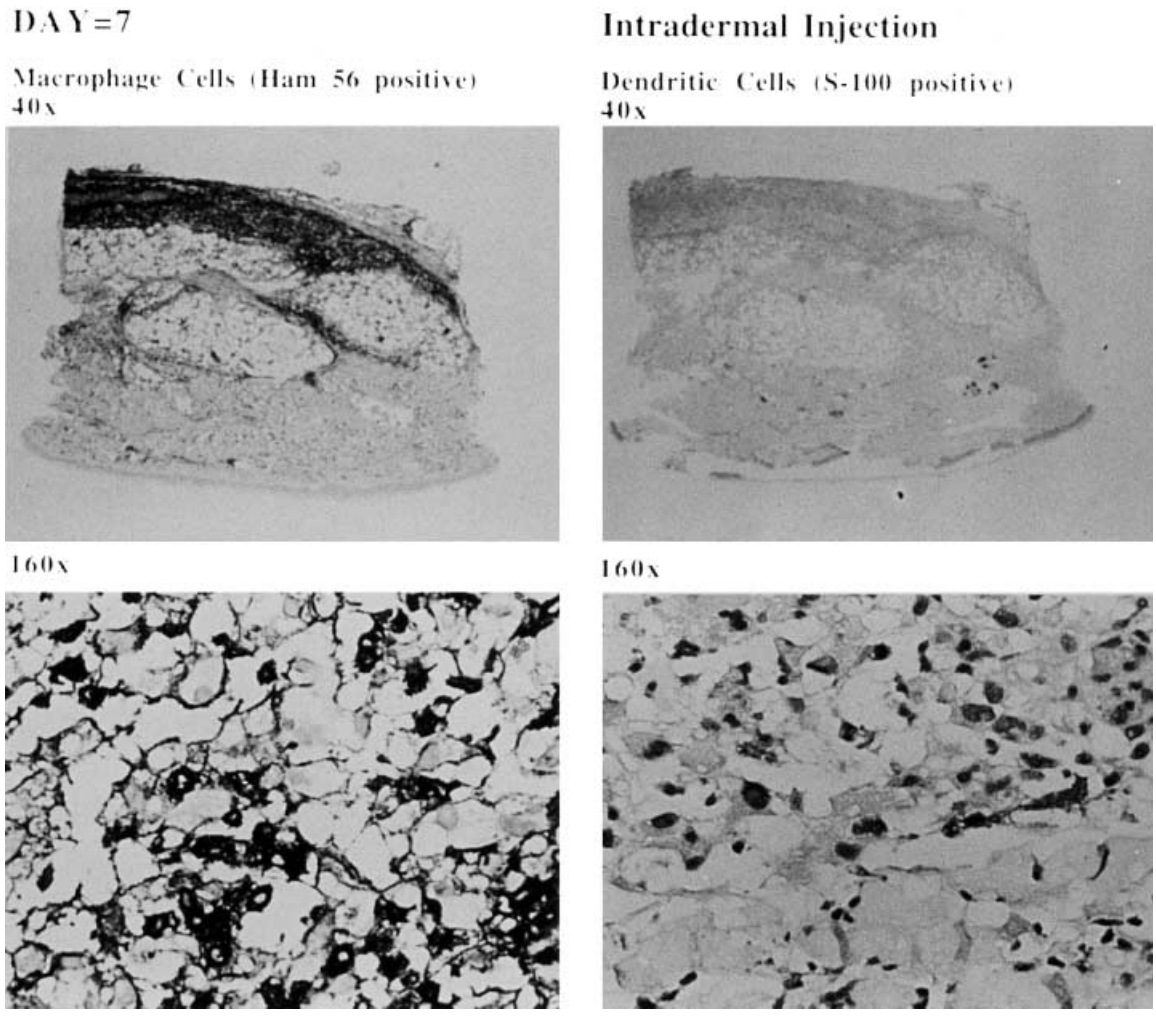


**Fig. 2** Biopsy of *MFG-GM-CSF*-transduced RCC vaccine site. Immunohistochemical staining reveals presence of macrophages and dendritic cells (DCs) at vaccine inoculation site. Dose level $2 = 4 \times 10^7$ cells

dose levels 2 and 3, with a trend toward higher degrees of DTH reactivity in patients receiving *MFG-GM-CSF*-transduced RCC vaccines than in patients treated with non-*MFG-GM-CSF*-transduced RCC vaccines [13]. DTH site biopsies from patients inoculated with *MFG-GM-CSF*-transduced RCC vaccines revealed infiltration by mononuclear cells and eosinophils, as well as perivascular cuffing of lymphocytes [13]. In general, such intense eosinophil infiltrates were not present in DTH site biopsies from patients treated with non-*MFG-GM-CSF*-transduced RCC vaccines. In the PCA trial, reactive DTH tests were present in two of eight patients before vaccine administration, while reactive DTH tests were evident in seven of eight patients after vaccination [14]. Reminiscent of DTH site biopsies obtained in the RCC trial, DTH site biopsies from vaccinated men in the PCA trial displayed perivascular cuffing by infiltrating lymphocytes and an accumulation of degranulating eosinophils, macrophages, natural killer cells, and T cells [14]. Some 80% of the T cells detected at the DTH sites expressed CD45RO by immunohistochemistry, indicative of activation by vaccination [8].

In clinical trials of cancer cell vaccines for melanoma, anticancer cell antibodies have been detected as a consequence of vaccine treatment [3, 15]. In our phase I trial of GM-CSF-secreting PCA vaccines, analyses of reactive DTH site biopsies disclosed evidence of activation of both Th2 and Th1, as well as $CD4^+$ T cell responses, with the accumulation and degranulation of eosinophils [14].

Although few if any B cells were present at vaccine sites or at DTH sites, we screened sera from men treated with GM-CSF-secreting PCA vaccines for antibodies directed at PCA antigens. In three of eight vaccinated patients, new antibodies, present at a titer of 1:1,000 by immunoblot analysis using LNCaP PCA cell extracts, were detected. The induced antibodies recognized antigenic polypeptides of 26, 31, and 150 kD [13]. Further characterization of the 150-kD antigen revealed that it was expressed by normal and neoplastic prostate epithelial cells and prostate smooth muscle cells, as well as by a number of human cancer cell lines, but not by prostate stromal cells, lung fibroblasts, or white blood cells [14]. The identities of the various antigens have not been ascertained.

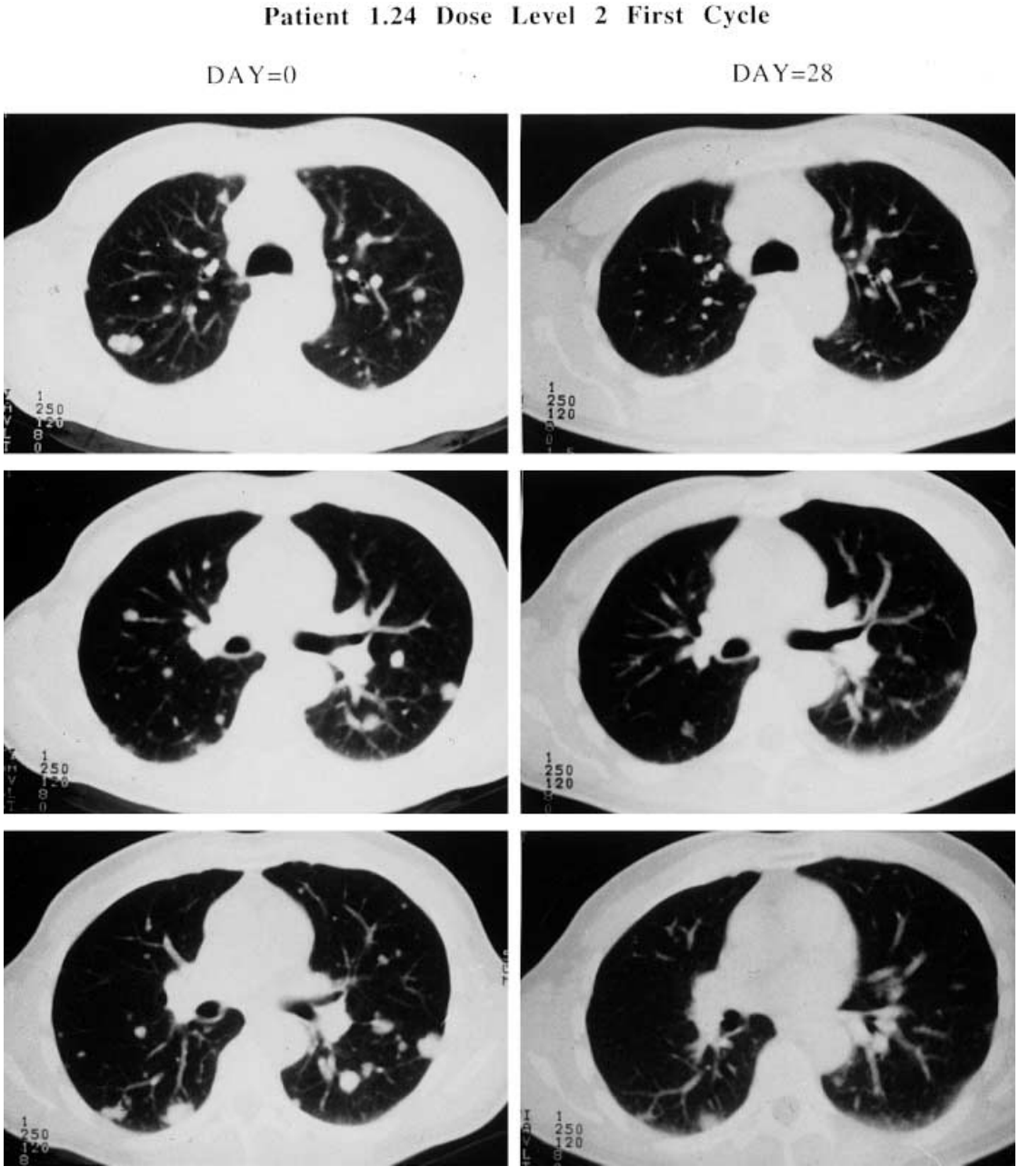


**Fig. 3** Computed tomography imaging of pulmonary metastases in a patient with RCC treated with *MFG-GM-CSF*-transduced RCC vaccine injections reveals a partial response as a consequence of treatment

While assessment of the clinical efficacy associated with GM-CSF-secreting cancer cell vaccine treatment was not the goal of either of our phase I clinical trials, some treatment response data were available from each of the vaccine series. In the RCC trial, one of the three patients treated at dose level 2 with *MFG-GM-CSF*-transduced RCC vaccines exhibited regression of multiple pulmonary metastases following treatment (partial response; Fig. 3) as evaluated using computed tomography (CT) imaging [13]. This patient had demonstrated progression of the same pulmonary metastases on previous CT images in the 2-month interval between nephrectomy and vaccine administration. The objective partial response lasted for 7 months and the patient ultimately died as a result of progressive RCC. In the PCA trial, all eight treated patients ultimately had progressively rising serum PSA levels [14].

## Discussion

These first clinical trials of CM-CSF-secreting cancer cell vaccines for RCC and PCA demonstrated that anticancer immune responses could be elicited by such vaccines in humans with minimal treatment-associated side effects. Reminiscent of preclinical observations using animal tumor models, the immune responses generated by

vaccination appeared likely to involve antigen-specific Th1 and Th2 CD4+ T-cell activation [6, 7]. The clinical trial data also hinted at the promise of vaccine immunotherapy for genitourinary cancers, since one of the RCC patients exhibited a partial response as a result of vaccine treatment. However, these first clinical trials also illustrate the technical difficulties associated with the preparation of autologous cancer cell vaccines: cancer cell recovery, expansion via propagation in vitro, and viability limited both the frequency with which vaccines could be created from resected tumor specimens and the quantity of vaccine cells (dose) available for administration.

With the "proof-of-principle" data collected in these first two clinical trials, we have focused future clinical development of GM-CSF-secreting vaccine treatment for genitourinary cancers on allogeneic as opposed to autologous cancer cell vaccines. Because paracrine GM-CSF secretion by dying cancer cells inoculated in the skin appears to activate skin DCs to prime anticancer immune responses [6], both GM-CSF-secreting autologous and allogeneic cancer cell vaccines elicit immune responses via autologous antigen presentation using host major histocompatibility complex molecules (Fig. 1). If the allogeneic cancer cells selected for vaccine construction contain a representative portfolio of cancer antigens, then allogeneic cancer cell vaccines might trigger anticancer immune responses as effectively as autologous cancer cell vaccines to critical cancer rejection antigens. Provocatively, antibodies appearing in one of the PCA patients treated with an autologous GM-CSF-secreting PCA vaccine recognized a 150-kD polypeptide antigen present in three established PCA cell lines (LNCaP, PC-3, and DU145). The major advantage of allogeneic cancer cell vaccines is that such vaccines can be prepared from established cancer cell lines via *GM-CSF* gene transfer, selected for high-level GM-CSF secretion, and manufactured in a nearly limitless supply. With such vaccines, since vaccine immunotherapy does not require surgery, a greater proportion of cancer patients will be candidates for treatment. Clinical trials of GM-CSF-secreting allogeneic cancer cell vaccines will be better able to ascertain the effects of different vaccine doses and schedules. In addition, clinical trials exploring the integration of vaccine immunotherapy into the multimodality treatment of RCC and PCA will be facilitated. Currently, clinical trials involving vaccination with irradiated GM-CSF-secreting LNCaP and PC-3 PCA cells are underway at a number of PCA treatment centers.

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