Dendritic Cell-Based Vaccination in Solid Cancer

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**Purpose:** Dendritic cell (DC)-based immunotherapy is rapidly emerging as a viable tool in cancer treatment. This approach has been used mostly in patients in the presence of defined tumor antigens such as melanoma. In this study, cancer patients with advanced disease that lacks defined tumor antigens were vaccinated with tumor lysate-pulsed DCs.

**Patients and Methods:** Twenty patients (pancreatic, hepatic, cholangiocellular, and medullary thyroid carcinoma) with stage IV disease were enrolled in the study. In 3-week intervals, freshly isolated autologous CD14 magnetic bead-selected monocytes were cultured in granulocyte-macrophage colony-stimulating factor and interleukin-4 to obtain immature DCs. These cells were pulsed with autologous tumor lysate and matured with tumor necrosis factor alpha. Mature DCs were applied into a groin lymph node, under ultrasound guidance. Adjuvant interleukin-2 (20,000 U/kg) was given subcutaneously daily, for 12 days, after each vaccination. Toxicity, tumor marker profile, immune response, and clinical response were determined.

**Results:** Vaccination was well tolerated. No physical signs of autoimmunity were detected. DC vaccination induced delayed-type hypersensitivity reactivity in 18 patients. Tumor marker responses were observed in eight patients. In addition, in three patients the generation of interferon gamma-positive T cells was induced during the vaccination. Objective changes in measurable lesions or tumor markers were evident in seven of 20 assessed patients. None of the patients was found to meet the criteria for partial or complete responses.

**Conclusion:** These data indicate that vaccination with autologous tumor-pulsed DCs generated from peripheral blood is safe and can induce tumor-specific cellular cytotoxicity. Clinical responses are achievable, even in patients with advanced disease.


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The lack of effective treatment modalities for many inoperable solid malignancies led to the search for new therapeutic options such as adoptive immunotherapy. In the past two decades, adoptive immunotherapy, based on tumor-infiltrating lymphocytes or lymphokine-activated killer cells, has been used in clinical trials.1-3 These early results gave first evidence that the manipulation of the immune system represents a promising tool in cancer immunotherapy.

Steinman et al4 discovered the crucial role of dendritic cells (DCs) for the induction of primary T-cell–dependent immune responses. DCs are now considered to be the best adjuvant for antitumor immunity. The possibility of obtaining large numbers of DCs in vitro has boosted research on their ontogeny and functions. The unique ability of DCs to take up, process, and present antigens, and to activate naive CD4+ and CD8+ T cells, makes them appropriate candidates for an experimental immunotherapeutic approach. Several in vivo studies in mice, as well as clinical phase I and phase II studies, proved the remarkable efficacy of immunotherapy with monocyte-derived dendritic cells (MODCs).5-8 However, clinical reports using DC vaccination protocols were mostly confined to melanoma and prostate cancer. One of the reasons for this limitation may be the fact that there are defined available peptide antigens for these entities. These antigens can be used to load DCs and to elicit a peptide-specific T-cell response in vivo. Defined antigens, however, are rare or unknown in many other tumor entities. It is therefore practical to use tumor lysate as a source of antigens to load DCs.

To optimize T-cell stimulation, “adjuvant” support of effector cells has been suggested by means of low-dose cytokine application. A promising effect of low-dose interleukin-2 (IL-2) application, combined with DC vaccination, was recently demonstrated in a mouse model.9,10

Based on these findings, we carried out a phase I study of combination therapy with autologous tumor lysate-pulsed DCs and low-dose IL-2 therapy in patients with incurable malignancies, including hepatocellular, cholangiocellular, and pancreatic cancer as well as not entirely resectable and metastasized medullary thyroid carcinoma. The aim of the study was to prove the safety and feasibility of DC immunotherapy in solid organ tumors.

**Eligibility Criteria**

Patients between 18 and 75 years, with stage 4 cholangiocellular, hepatocellular, pancreatic, or medullary thyroid carcinoma, were included in this trial. Patients were required to have an expected survival of 3 months or more; a Karnofsky index of 60% or more; normal or near normal renal, hepatic, and hematopoietic function; and to have received no chemotherapy, radiotherapy, or immunotherapy for at least 3 months before study enrollment. Only one patient received chemotherapy before DC vaccination. Patients with antibodies against human immunodeficiency virus (HIV)-1/2, human T-cell lymphotropic virus (HTLV)-1/2, or hepatitis B or hepatitis C virus, and patients with autoimmune disease, were excluded. Premenopausal females were required not to be pregnant and to take effective oral contraception. All patients included in the study gave written informed consent. The protocol was approved by the Institutional Ethics Committee and was conducted at the Departments of General Surgery and Radiology at University of Vienna Medical School, Vienna, Austria.

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the University of Vienna. Four to 10 DC vaccinations were administered at 21-day intervals on an outpatient basis.

**Clinical Protocol, DC Generation, and Immunization Schedule**

Generation of DCs from peripheral blood. Peripheral blood mononuclear cells (PBMCs) were isolated from 100 mL of EDTA whole blood by means of standardized density gradient centrifugation using Ficoll-Paque (Pharmacia, Uppsala, Sweden). Thereafter, CD14+ cells were separated by magnetic sorting, using VARIOMACS technique (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), according to the manufacturer’s protocol. Briefly, PBMCs were incubated with magnetic microbeads that were conjugated with monoclonal mouse antihuman CD14 antibody (anti-CD14 mAb). Twenty microliters of anti-CD14 mAb were added, per 107 PBMCs, and incubated for 15 minutes. Cells were washed and centrifuged at 300 × g for 10 minutes, supernatant was removed, and the cell pellet was resuspended and mounted on a magnetic column. Labeled and positively enriched cells were eluted from magnetic columns by removal from the magnetic device. Isolated CD14+ cells were cultured at a concentration of 1 × 10^6 cells/mL in standard culture flasks (Costar, Cambridge, MA) for 4 days in RPMI 1640/10% fetal calf serum medium supplemented with 5 mg/mL gentamicine at 37 °C. Thereafter, the culture was washed and reincubated for 36 hours. RPMI 1640, which contained 1,000 U/mL rh-GM-CSF and 1 ng/mL TNF-α, was added to the cultures at a concentration of 1,000 U/mL. On day 5, immature DCs were pulsed with autologous whole-tumor lysate.

Preparation of tumor lysate. Tumor samples from surgical resections were subjected to histological examinations and further processed to tumor lysate. Tumor samples (1 cm^3) were frozen in liquid nitrogen under aseptic conditions and were treated under laminar flow conditions and were treated under laminar flow by mincing with a scalpel and dissolving in phosphate-buffered saline (PBS). The samples were then lysed by freeze and thaw cycles. To avoid alteration of tumor peptides, enzymatic digestion was not performed. Samples were centrifuged at 2,000 rpm, and the supernatant was filtered using 0.45-µm pore-size filters. Cell lysis was verified by trypan blue staining, and the protein concentration was determined according to Bradford protein assay.

**Antigen pulsing, maturation, and application of DCs.** On day 5, tumor lysate was added to DC cultures at a final concentration of 100 µg/mL for 12 hours. Thereafter, the culture was washed and reincubated for 36 hours in RPMI 1640, which contained 1,000 U/mL rh-GM-CSF and 1 µg/mL tumor necrosis factor alpha (TNF-α) to promote DC maturation. Cells were then washed three times and finally dissolved in 300 µL PBS. An aliquot of the cell culture was tested for sterility (bacteriology and Gram’s staining) 2 days before application. Vaccine release criteria included a negative bacterial culture, negative Gram’s staining, and a fully mature DC phenotype that was confirmed by flow cytometry analysis. The cell suspension was injected into the patient’s groin lymph node under sterile conditions, using small-part ultrasound that was performed by a trained radiologist. Slow injection, under direct sonographic visualization, was done carefully to avoid disintegration of the lymph node. IL-2 was injected subcutaneously (SC) at a dose of 20,000 IU/kg for 12 days, beginning on the fourth day of each vaccination.

DC phenotype evaluation. The phenotype of monocytes and immature and mature DCs was determined by single- or two-color fluorescence analysis. Cells (3 × 10^6) were resuspended in 50 µL of buffer (PBS, 2% FCS, and 1% sodium azide) and incubated for 30 minutes at 4°C with 10 µL of appropriate fluorescein isothiocyanate or phycoerythrin-labeled mAbs. After incubation, the cells were washed twice and resuspended in 500 µL of assay buffer. The fluorescence was analyzed by an EPICS XL-MCL flow cytometer (Coulter, Miami, FL). For each sample, 15,000 events were acquired, and the percentage of positive cells was reported. Monoclonal antibodies specific for human CD1a, CD3, CD19, CD11c, CD14, CD40, CD80, CD86, CD83 (Immunotech, Vienna, Austria), and HLA-DR, as well as control immunoglobulin (IgG1 and IgG2a [Benton Dickinson, San Jose, CA]) were used to characterize DCs.

Delayed-Type Hypersensitivity Test. The delayed-type hypersensitivity (DTH) skin test was performed with tumor lysate-pulsed DCs and unpulsed DCs. After the fourth vaccination, and after the 10th vaccination when appropriate, pulsed DCs were intradermally injected into the forearm. A positive skin reaction was defined by a > 1.5-cm erythema and induration of the skin 48 hours after intradermal injection.

**Intracellular interferon gamma detection assay.** Intracellular staining for interferon gamma (IFN-γ) production of lymphocytes was performed as recently described. Brieﬂy, 5 × 10^6 CD14-depleted peripheral mononuclear cells, obtained before vaccination (T0) and after the fourth vaccination (T4), were cocultured with 1 × 10^6 mature tumor lysate-pulsed MDCs for 18 hours. Monensin (10 µmol; Sigma, Vienna, Austria) was added during the last 3 hours to block protein secretion. T0 and T4 cells, with and without exposure to tumor lysate, were used as controls. In a parallel set of experiments, 500 ng/mL of ionomycin (Sigma) and 50 ng/mL of PMA (Sigma) were added to the cell suspensions. Cells were harvested, washed, and permeabilized with a permeabilization agent (Immuntex), according to the manufacturer’s protocol. Cells were double-stained with phycoerythrin-labeled anti-CD69 or anti-γ-IFN and FITC-labeled anti-CD3-specific antibody (Immuntex). Appropriate IgG1 antibodies were used as isotype controls. Samples were analyzed on an EPICS XL-MCL flow cytometer (Coulter). The tests were performed before therapy was started and after the fourth vaccination was completed. Lymphocytes were obtained during each monocytes isolation procedure and stored in liquid nitrogen for further use.

Clinical monitoring. Adverse events were graded according to World Health Organization toxicity criteria. All patients underwent assessment of tumor status at baseline, and at 3, 6, 9, and 12 months after the first vaccination, using computed tomography scan or magnetic resonance imaging. Disease progression was defined as ≥ 25% increase in target lesions and/or the appearance of new lesions.

In all patients, tumor marker monitoring was performed at the beginning of the study and generally after each vaccination. Autoantibodies were analyzed before, and at least two times during, the vaccination period.

**RESULTS**

In accordance with the protocol, a total of 20 patients were enrolled in the study from April 1999 to November 2000, and they were vaccinated with autologous tumor lysate-pulsed monocyte-derived DCs. Patient characteristics are listed in Table 1. Nine patients with ductal pancreatic cancer, four patients with medullary thyroid cancer, two patients with hepatocellular carcinoma, four patients with cholangiocellular carcinoma, and one patient with adrenal cortical carcinoma...
participated in the study. A total of 141 vaccinations were performed (four vaccinations [six patients]; five vaccinations [two patients]; six vaccinations [two patients]; seven vaccinations [one patient]; eight vaccinations [one patient]; and 10 vaccinations [eight patients]). Two of 20 patients stopped treatment prematurely (patient no. 1, after the fourth vaccination; and patient no. 14, after the sixth vaccination).

**Phenotype of DCs**

Sufficient quantities of mature DCs could be generated from all patients (1 × 10^7 to 2 × 10^7, for each vaccination). They were analyzed, by flow cytometry, for the presence of antigens shown to be characteristic for monocyte-derived DCs. A representative example is given for all flow cytometry analyses performed for our cohort (Fig 1). Immature monocyte-derived...
dendritic cells (iMODCs) express HLA-DR, CD1a, CD11c, CD80, and CD40. They were found to be negative for CD14 and CD83 (Fig 1B). After pulsing and stimulation with GM-CSF and TNF-α, MODCs upregulate CD80, CD86, CD40, HLA-DR, and CD83, which has been shown to be a characteristic key feature of mature DCs (Fig 1C). Bacteriological testing and Gram’s staining were negative for all cell cultures.

**Toxicity**

DCs were carefully injected into a groin lymph node, under ultrasound guidance. All injections were successful in terms of targeting and maintaining lymph node architecture. No major toxicity occurred, and no side effects were observed at the intranodal vaccination sites. Four patients (one patient after the third vaccination and three patients after the fourth vaccination) developed elevated body temperature (\(< 38°C\)), which lasted for 1 to 2 days. Temporary exanthema developed in one patient (patient 9) 3 days after the fifth vaccination. The exanthema spontaneously disappeared without additional treatment after IL-2 withdrawal. At the site of IL-2 injection, a small itching induration was observed in 10 patients.

**Autoantibodies**

To investigate the autoantibody production during the observation period, serum levels of different autoantibodies were determined before, during, and after the treatment if the patient was still alive (Table 2). Autoantibodies were detected in the serum of four patients (two different autoantibodies in one

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**Table 2. Presence of Autoantibodies**

<table>
<thead>
<tr>
<th>Autoantibodies</th>
<th>Before Vaccination</th>
<th>After Vaccination</th>
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</thead>
<tbody>
<tr>
<td>Rheumatoid factors</td>
<td>1/20</td>
<td>1/16</td>
</tr>
<tr>
<td>Antinuclear antibodies</td>
<td>2/20</td>
<td>1/16</td>
</tr>
<tr>
<td>Antihistone</td>
<td>1/20</td>
<td>4/16</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>0/20</td>
<td>0/16</td>
</tr>
<tr>
<td>Anti-Ro/SSA</td>
<td>0/20</td>
<td>0/16</td>
</tr>
<tr>
<td>Anti-La/SSB</td>
<td>0/20</td>
<td>0/16</td>
</tr>
<tr>
<td>Anti-U1-RNA</td>
<td>0/20</td>
<td>0/16</td>
</tr>
<tr>
<td>Anti-sm</td>
<td>0/20</td>
<td>0/16</td>
</tr>
<tr>
<td>Antithyroglobulin</td>
<td>0/20</td>
<td>0/16</td>
</tr>
<tr>
<td>Anti-neutrophil cytoplasmic</td>
<td>0/20</td>
<td>0/16</td>
</tr>
<tr>
<td>Antithyroid</td>
<td>0/20</td>
<td>0/16</td>
</tr>
<tr>
<td>Anti-smooth muscle</td>
<td>0/20</td>
<td>2/16</td>
</tr>
<tr>
<td>Anti-parietal cell</td>
<td>1/20</td>
<td>0/16</td>
</tr>
<tr>
<td>Antimitochondrial</td>
<td>0/20</td>
<td>0/16</td>
</tr>
<tr>
<td>Anti-insulin</td>
<td>0/20</td>
<td>0/16</td>
</tr>
<tr>
<td>anti-pancreatic islet</td>
<td>0/20</td>
<td>0/16</td>
</tr>
</tbody>
</table>

**NOTE:** A: occurrence of autoantibodies in patients before and during vaccination; B: in those patients found to be positive for the presence of autoantibodies the highest values of autoantibodies are shown. Values of antinuclear, antiparietal cells, and anti-smooth muscle antibodies are given as titers. Values of rheumatoid factors and antihistone antibodies are given as U/mL.
patient) before starting therapy and in seven patients during therapy. One patient developed two different autoantibodies during therapy. The patients showed no clinical symptoms related to the autoantibody profile before or during the vaccination. The antibodies that were detected during therapy spontaneously returned to normal values a few weeks after the last vaccination in patients who received all 10 vaccinations.

**DTH Test**

To determine DTH reactivity, tumor lysate-pulsed DCs were injected intradermally into the forearm. All but two patients developed a positive DTH skin test after the fourth vaccination. In eight patients who were vaccinated 10 times, a second DTH skin test was performed after the last vaccination, and it was found to be positive.

**In Vitro IFN-γ Assay**

To assess the potential to generate a tumor-specific immune response, an assay was developed to determine intracellular IFN-γ production in peripheral T cells. During the trial, IFN-γ production in CD3+ lymphocytes was assessed by flow cytometry in patients 7, 8, 9, and 12. The results from patient 7 are shown as an example (Fig 2). After coculture with mature MODCs, the expression of CD69 and IFN-γ in CD3+ cells, which were not stimulated with PMA or ionomycin, was significantly higher in cells obtained after the fourth vaccination (DC/T4) than in cells obtained before vaccination was started (DC/T0). On stimulation with PMA and ionomycin during the coculture with mature MODCs, the expression of IFN-γ was significantly higher in DC/T4 than in DC/T0 cells, whereas expression of CD69 revealed no difference. T0 and T4 cells, with and without lysate pulsing, were used as controls, and they did not exhibit strong expression of either CD69 or intracellular IFN-γ. The results from patients 9 and 12 are similar to the results shown for patient 7. For patient 8, neither the expression of CD69 nor that of IFN-γ revealed a difference between CD3+ cells before and after the fourth vaccination (data not shown).

**Clinical Response and Tumor Marker Levels**

Serum levels of tumor markers, which are characteristic of the respective tumor entity of eight patients in whom a tumor marker response could be observed, are shown in Fig 3. In all patients with medullary thyroid cancer (patients 2, 5, 16, and 20), a tumor marker response could be observed (Fig 3A-3D). In patient 20, at the time of the initial surgical exploration, the tumor was infiltrating the esophagus and the trachea. The patient suffered from recurrent bronchitis, and he had problems swallowing. During the vaccination treatment, the symptoms disappeared, and to date, the patient remains free of these symptoms. Restaging procedures, 3 months after starting DC therapy, revealed a partial regression of the mediastinal tumor mass (Fig 4A and 4C). Thirty months after starting DC therapy, the patient developed metastases in the femur and in the pubic bone and died 2 months later. In patient 5, the decrease of the tumor levels of tumor markers was observed.
markers also correlated with a partial decrease of the cervical tumor mass (Fig 4B and 4D). The disease progressed, and the patient died 9 months after starting DC therapy. In patient 16, the tumor marker levels remained almost constant during the observation period. Restaging analysis of this patient revealed stable disease. The patient is still alive, 24 months after starting DC therapy. Patient 2 died 4 months after starting the therapy, with rapid tumor progression; however, a decrease of calcitonin serum levels could be observed after the first 3 months (Fig 3A). In patient 4, with hepatocellular carcinoma, tumor markers remained constant over a period of 6 months. He survived 18 months after initiating the therapy (Fig 3E). In one patient (6) with multilocular cholangiocellular carcinoma, a decrease in the tumor marker profile could be observed, and he survived 5 months after the initiation of therapy (Fig 3F). In patient 9, the histological specimen obtained during the operation was classified as hepatocellular carcinoma. Several weeks after study enrollment, the diagnosis was modified to metastasized adrenal cortical carcinoma. In this patient, a decrease in the tumor marker levels was also observed 6 months after starting the therapy (Fig 3G). The local tumor mass at the site of the left adrenal gland increased from 16 to 18 cm in the last 4 months. The patient died 9 months after beginning the therapy. In patient 12 (Fig 3H), a ductal adenocarcinoma, localized in the cauda of the pancreas, was radically resected by distal pancreatectomy. Although radical resection (RO; with free margins and no evidence of residual tumor) resection was achieved, 3 months after the first operation, the patient developed recurrent disease.
in the caput of the pancreas, and DC therapy was initiated. The tumor marker levels in this patient increased slowly during the observation period, but they were stable in the last 2 months. The patient survived for 12 months after the initial diagnosis was made. In one patient (patient 7) with cholangiocellular carcinoma that was localized in both liver lobes, no tumor marker could be determined. However, reevaluation revealed that in the right liver lobe, parts of the tumor bulk regressed, and the disease was classified as stable during the last 3 months. The patient is still alive and in good clinical condition, 22 months after starting the therapy.

DISCUSSION

In contrast to other clinical studies, the presented DC vaccination protocol was based on magnetic bead selection to obtain highly purified MODCs. We demonstrated that this method is safe and that it guarantees a large number of purified monocytes for in vitro generation of DCs, even from patients with advanced tumor disease.

In this study, tumor lysate-pulsed DCs were stimulated with TNF-α to obtain fully mature DCs before vaccination. Recent data support this approach because it was demonstrated that mature DCs are more potent in inducing and sustaining a specific cytotoxic T-cell response immune response.14,15 It became evident that immature DCs are actively endocytotic and can internalize, as well as process, exogenous antigens efficiently, and may therefore be most suitable for the delivery of antigens.16,17 In contrast, mature DCs, with high expression of major histocompatibility complex (MHC) class 2 and costimulatory molecules, are of critical importance for the induction of tumor-specific cytotoxic T-cell–mediated immunity.13,18 In this context, it has been shown that immature antigen-presenting DCs, which express low levels of MHC class 2 molecules and quite low levels of costimulatory signal molecules, induce immunotolerance rather than immunity.19 We therefore added TNF-α to tumor lysate-pulsed DCs for final maturation, which resulted in a homogenous CD83+ DC population at the time of vaccination (Fig 1C).

However, it was uncertain whether the advantage of such highly stimulatory DCs would be counterbalanced by the possibility to induce autoimmune disease. In our study, we observed a transient increase of autoantibodies in several patients (Table 2). However, no patient developed any clinical symptoms.

In contrast to other studies in which a variety of specific shared tumor-associated antigens were applied, we had to use crude tumor lysate for pulsing DCs because of the lack of available defined tumor antigens in these distinct solid tumors. It has been demonstrated previously that DCs pulsed with whole protein may be more effective than DCs pulsed with MHC class 1 restricted peptides for eliciting antigen-specific immune responses. This may be related to the wide repertoire of different antigens present in the lysate.20,21 Among the five different tumor entities, it is noteworthy that pancreatic cancer tissue specimens contain a relatively low number of cancer cells. This fact might lead to tumor lysates with a comparably small number of tumor antigens. The question of whether this has a negative effect on the quality of the therapy has yet to be evaluated. To date, it is not known how much actual tumor antigen is necessary to induce a clinically relevant specific antitumor response.

For the route of administration, we used direct intranodal injection as described by Nestle et al.5 We chose this approach because there is growing evidence that intranodal injection may be favorable to other forms of application, such as intravenous or intradermal injection.22 Using a small-part ultrasound set, it could be demonstrated that it is easy to apply the vaccine directly into the lymph node. This form of application was well tolerated in all patients, and it was routinely performed in an outpatient setting.

Shimizu et al9-15 reported the beneficial adjuvant effect of low-dose IL-2 administration in mice, in combination with DC treatment. This formed the rationale to administer IL-2 SC, at a dose of 20,000 U/kg, for 12 consecutive days after each vaccination. We could not, however, detect any alteration of the peripheral blood lymphocyte subpopulations, measured by flow cytometric analysis, as a result of IL-2 administration (data not shown), which might be explained by the extremely low dosage of IL-2. To what extent the administration of IL-2 had a beneficial effect, concerning the stimulatory capacity of DCs in this study, cannot be concluded with certainty.

The primary aim of this study was to assess the feasibility and toxicity of adoptive immunotherapy using mature tumor lysate-pulsed DCs and additional administration of low-dose IL-2 in patients with incurable malignancies. This clinical trial demonstrated that the administration of magnetic bead-selected MODCs leads to no major toxicity and is feasible and safe, even in patients with advanced tumor disease. There was no clinical evidence for the development of an autoimmune disease. Furthermore, the therapy was well tolerated and could be performed on an outpatient basis.

In addition, we evaluated the eventual clinical responses and explored the therapeutic potential of this method in different entities of solid organ malignancy. In several patients, we observed objective changes in measurable lesions or tumor marker levels. This is particularly remarkable, given the far-advanced clinical stage of the disease and the current lack of any conventional treatment options for these patients. However, none of our patients was found to meet the formal criteria for partial or complete responses. Nevertheless, patients responded dramatically well, especially the medullary thyroid cancer patients. Together with recent in vivo and in vitro investigations, we
suggest that medullary thyroid cancer is particularly suited for DC-based immunotherapy.\textsuperscript{23,24}

In this clinical trial, only patients with advanced tumor stage were treated. However, minimal residual disease may be the optimal clinical setting to apply such a noninvasive and nontoxic therapeutic approach. We currently perform an early phase III prospective randomized trial in radically resected cancer patients who have a high risk of relapse. Several aspects of vaccine optimization, antigen preparation, and method of application are the foci of ongoing and forthcoming studies. If the initially promising results presented here are confirmed, DC-based immunotherapy could be considered for patients in earlier stages of disease.

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