Dendritic Cell Vaccines for Cancer Therapy: Fundamentals and Clinical Trials

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Contents

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19.1 Introduction

Mobilization of the immune system for the generation of an effective lymphocyte response against tumor tissue is one of the main goals of immunotherapy. It implies the necessity of a coordinated participation of the innate and adaptive immunity mechanisms in order to both trigger an effective response against tumor cells and preserve the host from autoimmune response. In this aspect, dendritic cells (DCs) perform a fundamental role in linking the innate defenses to the specific responsiveness by lymphocytes.

The very first report on DCs was published in 1868 by Paul Langerhans who found branched skin cells by gold staining (called Langerhans cells), whose "dendritic" extensions of plasmatic membrane resembled nervous cells [1]. A century later Prunieras [2] coined the expression "dendritic cells" for the Langerhans cells and proposed that they can capture antigens and are involved in primary defense against pathogens. However, the key contribution toward the morphological, phenotypical, and functional identification and classification of DCs as a new population of leukocytes was given by Steinman and Cohn, whose seminal reports from 1973 to 1978 are considered the beginning of a new era in this research field [3–7].

There are two main DC populations: the conventional DC, a myeloid-derived cell lineage, and the plasmacytoid DC (pDC), a lymphoid-derived lineage [8]. Although these two popula-

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tions can be differentiated by morphological and surface markers, each DC type shows a wide phenotypical variation and multifunctional role in the immunosurveillance and regulation of the immune system [9, 10]. Thus, conventional human DC express CD4, CD11c, and CD1a or CD83 and the MHC class I [11, 12]. Maturation/activation of these cells is characterized by the expression of CD80, CD86, CD40, and CCR7 [8]. Differently, lymphoid pDC are featured as $CD4^+/CD1a^-/CD11c^-/CD123^+$ cells [13].

DCs are the main professional antigen-presenting cells (APCs) and perform a continuous surveillance and recognition of the microenvironment of tissues and organs where they are found as immature cells (iDCs). In this condition, they have high capacity for capturing soluble and particulate antigens by endocytosis, phagocytosis, and micropinocytocis [3, 11, 14, 15]. The intakes of opsonized and non-opsonized antigens can be mediated by several surface receptors such as FcγR [11], mannose receptor (MR) [16], DC-SIGN [17], type C lectin receptors (DEC-205) [18], as well as *Toll*-like receptors [12, 19]. These antigens are then processed into peptides that are subsequently presented to T lymphocytes in the context of the major histocompatibility complex (MHC) [11, 12, 20].

Immature DCs do not have the unique ability for stimulating naïve T cells, since in this state they do not have the co-stimulatory signals required for T-cell activation. Considering that contact between iDC and a specific T cell can drive lymphocytes to cell anergy or induce regulatory cells [21, 22], DC maturation is critical for achieving the balance between effector responsiveness and autotolerance [11].

Proinflammatory signals induce not only the migration of iDC to the secondary lymphoid organs but also their maturation and activation. In contrast to iDC, mature DCs show reduced endocytic and antigen processing ability, while becoming highly efficient presenters of processed antigens for lymphocytes at the T-cell sites of lymphoid organs. Mature DCs express a higher density of CCR7 that drives their chemotactic migration toward the T-cell sites [11, 23].

Maturation is also followed by increased expression of a set of the abovementioned surface markers and by production of several proinflammatory cytokines, such as IL-12, IL-18, TNF-α, IL-23, IL-10, and IFN-α, depending on the stimulating factor [24–26].

Phenotypical and cytokine profile of mature DC contribute to the recruitment, interaction, and activation of lymphocytes for the development of an efficient response against pathogenic microbes, allergens, and allogeneic tissues [27, 28] and were also evidenced in antitumor response [8]. In fact, it was reported that tumor mass-infiltrating DCs are usually suppressed or maintained as iDC in situ. These observations have instigated many authors to try to stimulate infiltrating DCs to play a more effective role against tumor cells [29, 30] or to transfer autologous or allogeneic DCs after *in vitro* loading with tumor antigens, thus giving rise to several studies on the feasibility of using DC as therapeutic vaccines for active immunization of cancer patients.

Such studies have benefited from the observation that murine DC can be differentiated *in vitro* from bone marrow precursors. Further investigations were strongly reinforced by the finding that human DC could be differentiated from peripheral blood monocytes through treatment with adequate cytokine cocktails, usually a combination of IL-4 and GM-CSF [8, 31–34].

Being the main professional antigen-presenting cells, DC constitutively express both MHC class I and class II antigens on their surface. This feature is closely associated with their effective antigen-presenting function, whereas strategies for improving the expression of these molecules have been proven to enhance the antitumor response triggered by DC vaccines. In this aspect, it was early observed that increasing the expression of MHC class II molecules on DCs by transfecting them with MHC class II transactivator genes (*CIITA*). It induces four times more CTL than parental untransfected DC or DC transfected with irrelevant genes [35].

In an early report, even before the flourishing of proposals for DC-based antitumor vaccines (DC vaccine), it was observed that monocyte-derived phagocytic cells could be sensitized by

apoptotic bodies obtained by dead tumor cells [36]. Current studies are still using peripheral blood cells to generate human DC and bone marrow cells for murine ones; however, the efficiency of these vaccines appears to be dependent on a number of factors including generation of mature DCs [37–39], sustained production of IL-12 [40–43], and overcoming the suppressive microenvironment provided by regulatory T cells [37, 44–47] and myeloid-derived suppressor cells [48–51]. In fact, there is a variety of approaches to generate DC vaccines and it has been observed that each type of tumor has particular features that can hinder the effectiveness of such preparations.

19.2 Strategies for Developing Clinical Grade DC Vaccines

One of the main issues for generation of clinical grade antitumor DC vaccines is the choice of the technique for DC loading with tumor antigens. They range from the easier antigen preparation of tumor cell lysates by quick freeze-and-thaw cycles to the generation of tumor-DC hybrid cells or their transfection with tumor nucleic acid. However, there is still no definitive agreement on what strategy is the best.

Results with DCs loaded with lysates of tumor cells are controversial since some studies have shown that this approach results in a poor protective role of DCs, whereas other authors have successfully prepared them. Some details can be crucial to the effectiveness of lysate-pulsed DC vaccines. For instance, [52, 53] inhibitory effect of lysate on DC maturation can be reduced when tumor cells are stressed by heating at 42 °C for 25 min prior to the cell lysate preparation. It is hypothesized that the expression of heat shock proteins (HSPs) by tumor cells can avoid the suppressive effect of cell lysate by increasing DC maturation, an observation corroborated by others [54–56]. Induction of HSPs may be a required feature for increasing the immunogenicity of tumor cells by treatment with chemotherapeutic agents. The authors have observed that low nontoxic concentrations of paclitaxel or doxorubicin are able to alter the expression of a number of genes including HSP70, HSP40, and HSP105 mRNA [53].

Aiming to compare different methods for loading DCs with tumor antigens, it was observed that lysate obtained from a homogenate of solid tumor cells exerted a poor effect on the ability of DCs to stimulate antitumor activity [57]. Stressed tumor cells were obtained by freeze-and-thaw cycles or by irradiation at 30 Gy, with the irradiation being more useful than a freeze-and-thaw process. However, the best method for loading DCs in the mentioned study was their fusion with live tumor cells. The authors observed that irradiation of tumor cells at 30 Gy was effective at blocking their proliferative ability and did not affect their usefulness in preparing tumor-DC hybrids. For clinical purposes, loading DCs with tumor-associated proteins or peptides has been preferred in relation to the total tumor lysates. In a phase I study, patients with advanced melanoma were vaccinated with $CD34^{+}$-derived DC pulsed with melanoma peptides. Some patients showed peptide-specific DTH response, as well as Melan-A- and gp-100-specific CTL in the peripheral blood [45].

One of the limitations of preparing DC vaccines pulsed with tumor lysate is that the available tumor tissue is usally not sufficient for repeated applications for the patient. The use of tumor RNA for encoding tumor antigens was first proposed by Nair and Gilboa's group [58, 59], and there is substantial evidence that RNA transfection is a superior method for loading antigens onto DC [60–62]. An important point to consider is that tumor RNA can be amplified through molecular biology techniques, so that even a small amount of original RNA can be employed to obtain sufficient material for DC loading. Moreover, both total RNA and selected sequences can be used for DC-pulsing in order to drive the antigen presentation toward a more specific immune response. Finally, RNA shows a safety advantage on DNA, since it cannot be permanently integrated into the host genome.

The strategy of DC transfection with CEA RNA has been used both in murine [63, 64] and human systems [59, 65, 66]. Sakakibara et al.

[67] have proposed a method for generating DC vaccines more rapidly by incubating monocytes with GM-CSF and IL-4 for 24 h (fast DC) transfection with tumor mRNA and cultivation with a maturation cocktail for an additional 48 h. The authors observed that mature fast DCs and standard DCs displayed comparable levels of many markers expressed on DCs, including HLA-DR, CD83, CD86, CD208, and CCR7. Both were equally able to elicit specific T-cell response and IFNγ-secreting T cells, leading to the conclusion that mature fast DCs are functional antigen-presenting cells (APCs) capable of inducing primary T-cell responses.

Vaccination with tumor-DC hybridomas using autologous melanoma or renal carcinoma cells and allogeneic DCs is able to change the natural history of the disease, since it may present stabilization [31] or even regression of metastatic lesions with local fibrosis [68]. Whether a patient was unable to fight the tumor development, it is probable that his/her own DCs were unable to efficiently process and present relevant tumor antigens to generate specific CTLs. The fact that most tumor antigen peptides are considered to be self-antigens hampers the generation of an effective CTL response. This point of view has led some authors to suggest the use of allogeneic or semi-allogeneic systems to generate DC vaccines. Fusion of allogeneic DCs with autologous metastatic colon cancer cells was able to activate both $CD4^+$ and $CD8^+$ T cells in just 24 h, in a higher number than controls, while $CD8^+$ cells were significantly able to lyse target cells [69]. It can also solve some practical problems, namely, (a) it is usually possible to generate a limited number of samples of autologous DCs for vaccination, whereas a higher number of DCs could be generated from healthy allogeneic or semi-allogeneic donors; (b) the cellular reactivity triggered by allogeneic or semi-allogeneic DCs for allogeneic MHC antigens could facilitate the elimination of escaped tumor variants, as happens in the recipients of semi-allogeneic bone marrow transplantation; and (c) autologous tumor cells are sometimes scarce, which may be overcome by the use of stable tumor cell lines as the source of allogeneic tumor antigens for pulsing autologous DCs.

Evaluation of the efficiency of syngeneic, allogeneic, and semi-allogeneic DCs has shown that hybrid cells prepared with allogeneic or semi-allogeneic DCs were more effective than syngeneic ones and also worked better as therapeutic vaccines, thus protecting hosts against pulmonary metastasis. Actually, allogeneic and semi-allogeneic DCs more effectively induce CTL activity, as well as NK cytotoxicity, and induce higher levels of IFN-γ, as well as the IFN-γ:IL-10 ratio [70].

The use of exosomes for DC loading has also been proposed by some authors [71–74]. Exosomes are defined as constitutive nanovesicles that can be exocyted by both tumor and DCs displaying a sample of all membrane molecules of original cells [75, 76]. It was observed that vaccination with tumor peptides is more effective when carried on exosomes [72, 77]. Dai et al. [54] revealed that these nanovesicles can be isolated from heat-stressed tumor cells, culturing them for 43 h at 37 °C, followed by incubation for 1 h at 43 °C. After purification by ultracentrifugation on a discontinuous density sucrose cushion, exosomes were used to induce maturation of monocyte-derived DC. DCs loaded with such nanovesicles showed strong upregulation of HLA-DR, CD86, and CD40, as well as the production of IL-12p70 and TNF-α. This technology can also be used for increasing the immunogenicity of tumor cells, since they are able to uptake mature DC exosomes and express themselves, thus activating molecules such as HLA-DR and CD86 [78].

Cross-priming performed by DC is a phenomenon that can enhance the transference of antigenic peptides through HSP, such as gp96 and HSP70 [79–81]. Some HSPs obtained from tumor cells seem to be loaded with tumor antigens and can be internalized by DC through phagocytosis receptors. Such peptides can further be presented in the MHC class I context for inducing $CD8^+$ response and subsequent specific attack toward tumor cells [82–85]. Although the use of HSPs seems to represent a good strategy for enhancing the DC loading with tumor antigens [86–88], the clinical application faces some limitations including the difficulty to construct

the HSP-peptide complex and the necessity of a large amount of antigen source for obtaining a sufficient quantity of purified HSPs [89].

19.3 Routes of Administration

Another fundamental aspect of DC-based immunotherapy is the route of choice for administrating ex vivo prepared DCs. Clinical trials have reported various routes of DC administration, aiming to achieve an efficient delivery of cells to the appropriate immune site. Therefore, DCs can be inoculated by intradermal (i.d.), subcutaneous (s.c.), or intranodal (i.n.) routes to deliver loaded cells to regional lymphoid tissues, whereas intravenous (i.v.) methods should be chosen for their systemic distribution. There are also a number of studies showing the feasibility of intratumor (intralesional) inoculation of DC vaccines.

In vivo tracking of s.c.- and i.d.-inoculated DCs in multiple myeloma patients revealed their migration to the regional lymph nodes [90]. In fact, the i.d. route seems to be more efficient than s.c. for cell delivery to lymph nodes of patients with metastatic diseases [91]. Although these routes lack DC migration to the spleen, they appear to be more effective for inducing specific antitumor responses compared to the i.v. method [92, 93]. Tracking studies have also revealed that i.v. inoculation promotes DC distribution to the liver, spleen, lungs, and bone marrow. It was observed that DCs accumulate in the spleen just 3–24 h after inoculation [92]. Since the majority of relapsing diseases result from metastatic tumor cells, it is reasonable to infer that systemic distribution of DCs to the main targets for metastasis (lung, liver, and bone marrow) would be preferred in the protocols developed for preventing them [94–96].

Despite the suppressive microenvironment established at the tumor site, intralesional administration of DC was shown to be feasible, safe, and well tolerated [97–99]. Of course, this choice is limited by the tumor accessibility, while Mirvish et al. [100] suggest that in some cases the combination of different routes should be necessary for achieving successful immunization.

Considering the different designs for tumor antigen delivery, as well as the different administration routes, in the next section we will highlight the clinical experience in relation to selected diseases.

19.4 DC Vaccine for Prostatic Cancer

Prostate cancer is the second most frequent type of neoplasia worldwide, accounting for more than 903,500 new cases each year [101]. Most patients are successfully treated by prostectomy or radiotherapy, but about 30 % of them relapse [102]. In this aspect, immunotherapeutic approaches became an attractive alternative treatment, particularly for patients with the advanced disease, since the conventional treatments are merely directed against the symptoms. In addition, its feature of slow progression facilitates the manipulation of the immune system in order to enhance the recognition of tumor antigens.

The first DC vaccine approved by the U.S. Food and Drug Administration (FDA) for cancer therapy targets prostate cancer [103–105]. This vaccine, called *sipuleucel-T* (Provenge® – Dendreon, Seattle, WA, USA), was developed for castration-resistant metastasis of PC (for both symptomatic and asymptomatic patients). It is a DC-enriched autologous cell suspension from the own patient prepared by culturing them with a fusion protein called PA2024, which is constituted by the granulocyte-macrophage colony stimulating factor (GM-CSF) and the prostatic acid phosphatase (PAP) widely expressed by tumor cells [105–107]. The analysis of disease progression and overall survival in two phase III studies (D9901 and D9902A) showed that this vaccine was able to increase the overall survival from 4.5 to 6.7 months [104, 105].

A third phase III trial has shown that *sipuleucel-T* improved patient survival time by 4.1 months, showing a 22 % lower relative risk of death than the placebo group [103]. Another positive result of these trials is that patients have

shown variable reduction of PSA levels (prostatic-specific antigen), the main prognostic marker of this disease [104, 108].

The cellular immune response was also improved by treatment with *sipuleucel-T*, with 73 % of patients presenting an adequate lymphoproliferative response, whereas merely 12 % of the placebo group showed similar responsiveness [103]. In addition, generation of PAP-specific T lymphocytes was significantly higher in vaccinated patients than in those receiving placebo (27.3 % *vs*. 8.0 %), while minimal and well-tolerated collateral effects were also observed [106, 109].

In another successful approach, prostatectomized patients with biochemical relapse were treated with autologous DCs pulsed with human recombinant PSA (Dendritophage-rPSA) [110, 111]. Nine out of twenty-four patients showed 50 % reduction in PSA levels, whereas 11 others showed less pronounced diminution (6–39 %). In addition, 13 patients showed PSA-specific T-lymphocyte responsiveness. Six of the patients did not present any sign of circulating tumor cells during a 6-month follow-up. These results are favorable since handling patients with biochemical relapse is still a challenge for oncologists, urologists, and radiotherapists, due to the difficulty of ascertaining the correct location of relapsing disease.

Considering the difficulty of obtaining sufficient amounts of tumor antigens, Fong et al. [111] have proposed the use of xenogeneic murine PAP for loading autologous DCs. Six out of twenty-one patients with metastatic prostate cancer showed stabilization of the disease, with no rise of PSA levels nor the development of PSA-specific T cells.

Preparation of tumor-DC hybrid cells was also tested in prostate cancer. Hybridomas prepared with three different cell lines successfully induced an *in vitro* response in a mixed leukocyte culture by enhancing the IFN-γ production. Results were especially evident when ONYCAP23 and LNCaP were used for fusion (73 % and 67 %, respectively). Interestingly, the ONYCAP23 based hybridoma have induced specific T-cell response to different tumor targets [112].

A phase I/II study using DCs pulsed with allogeneic tumor cell lysate has demonstrated good tolerance and absence of toxic effects. However, although some patients have presented significant *in vitro* proliferation of specific antitumor lymphocytes, this approach has not achieved relevant clinical results [113].

19.5 DC Vaccine for Melanoma

The first clinical study on DC vaccines in melanoma patients was published by Nestle et al. [114], who analyzed the efficacy of DCs pulsed with HLA-A2-restricted peptides and autologous tumor cell lysates. Two out of six patients presented complete response to vaccination, while four of them developed specific DTH response.

The use of allogeneic tumor cell lysate for loading DCs, assessed in a phase I/II study, found that only 1 out of 15 patients with melanoma treated with autologous iDC pulsed with tumor lysate showed complete remission of metastasis. When the follow-up was discontinued, this patient had maintained an asymptomatic condition for 24 months [115].

More recently, melanoma patients were treated with DCs pulsed with melanoma peptides (HLA-A2$^+$) or tumor lysates (HLA-A2$^-$), in association with IL-12, celecoxib, and metronomic doses of cyclophosphamide (phase II study). This association was well tolerated by patients, and 29 % of patients with metastasis had the disease stabilized for 7–13.7 months. These patients also showed a higher median overall survival than patients with progressive disease (10.5 *vs*. 6 months). No significant difference of efficacy was observed between DCs loaded with cell lysate and peptides, although no correlation was found between the development of specific immune response and clinical response [116].

The use of autologous tumor RNA for loading autologous DC has promoted increased numbers of IFN-γ-producing CD4$^+$ cells [117]. This result merits attention because the strategy of using RNA aims to stimulate CD8$^+$ response since it implies the generation of tumor peptides at cytoplasm, which would be processed through

the cytosolic machinery. Thus, the expected effect on the activation of $CD4^+$ cells can favor the establishment of memory $CD8^+$ cells (Shedlock and Shen 2003; Janssen et al. 2003). In a phase I/II study, Kyte's group showed that administration of RNA-pulsed DCs was able to induce a specific DTH reaction and *in vitro* lymphoproliferative responsiveness as well as IFN-γ production [118].

Cell fusion technology was also applied to melanoma and kidney cancer patients, by fusing autologous tumor cells with allogeneic DC obtained from healthy donors [31, 119]. The measurable clinical response from these patients demonstrated that the disease had been stabilized for a median of 6 months, with no relevant collateral effects [31].

19.6 DC Vaccine for Colorectal Cancer

DCs are constituent cells of lamina propria and are involved in every local pathological condition. Mechanical disaggregation and enzymatic digestion of intestine specimens of patients with different types of colon disease – including colorectal cancer, Crohn's disease, ulcerative colitis, and nonmalignant, noninflammatory conditions – show that DCs correspond to 2 % of cells isolated from lamina propria [120]. As to the ability of these cells to stimulate lymphocyte activity, DC-rich suspension induces mixed lymphocyte response (MLR) by T cells. However, tumor-infiltrating DCs poorly stimulate T lymphocytes in a primary allogeneic culture (MLR) and are not able to induce significant levels of IL-2 or IFN-γ [120].

The C-type lectin DC-SIGN (DC-specific intercellular adhesion molecule-3-grabbing nonintegrin) is involved in the recognition of colorectal cancer cells by DCs [121]. Immature DCs within colon tumor tissue expressing DC-SIGN, but not mature DCs, interact with tumor cells by binding to $Lewis^x$ and $Lewis^y$ carbohydrate of CEA in tumor cells. Interestingly, DC-SIGN do not interact with CEA expressed by normal colon epithelium that shows low levels of Lewis epitopes. Therefore, DCs interact with human colon SW1116 tumor cells that express aberrantly glycosylated Lewis epitopes (Le^a/Le^b) of CEA and CEA-related cell adhesion molecule 1 (CEACAM1), an interaction that induces the production of immunosuppressive cytokines such as IL-6 and IL-10 [122].

Immunohistochemical analysis of infiltrating cells showed that mature $CD83^+$ DCs are found in almost all primary colon carcinoma samples and in some metastases. Heterogeneous infiltration patterns vary from diffuse cells to clustered DCs that tend to accumulate around vascular structures and the marginal zone of lymphoid aggregates [123]. Data on maturation markers on DCs that infiltrate primary tumors are contradictory. Indeed, some authors observed that around 90 % of $CD83^+$ cells were double-stained by anti-CD40 or anti-CD86 antibodies, indicating their *in vivo* activation [123], whereas others reported that 64–97 % of cells do not express B-7 molecules [124, 125], even after stimulation with TNF-α, IL-4, and GM-CSF [125]. The density of DCs at the tumor site was higher in patients with a high proportion of activation markers (CD86 and CD40), suggesting that mature DC can actively migrate to or be activated in the tumor microenvironment under exposure to tumor antigens [123].

Immunization of patients with DC vaccine in phase I/II clinical trials showed that the vaccine was effective for 16.7 % of patients in the phase I study and for 23 % of them in the phase II study [59]. Messenger RNA of TAT protein transduction domain and calreticulin increase the immunogenicity of CEA and the effectiveness of mRNA-pulsed human DCs. It is interesting that transfection of DCs with calreticulin mRNA seems to be associated with activation of $CD4^+$ T cells, whereas TAT protein mRNA preferentially stimulates $CD8^+$ cells [126]. Since mRNA represents only up to 5 % of total cell RNA, *in vitro* amplification of mRNA was shown to be feasible for producing immunogenically active CEA-encoding mRNA [65].

Instead of using mRNA for known specific antigens such as CEA and HER2/neu, DCs transfected with total tumor RNA were able to induce

CTL response, while effector cells were able to recognize both the original tumor cell line used for RNA preparation (SW480) and other cell lines, namely, HCT-116 (colon cancer) and A498 (kidney cancer) [127]. Supporting this strategy, a clinical trial using total RNA extracted from metastasis tumor cells for pulsing autologous DCs, followed by inoculation in the patients (four injections, every 4 weeks), showed an ability to induce specific T response to CEA [128].

Analysis of ten clinical samples of colorectal carcinomas showed that 60 % of them overexpressed the antigen EphA2 [129]. Murine DCs pulsed with human EphA2 were observed to induce antitumor response against EphA2-transfected MC38 cells. Results have shown that Eph-DC strongly delayed the tumor growth and induced specific $CD8^+$ cells and $CD4^+$ cells which play a critical role in the antitumor response.

19.7 DC Vaccine for Nervous Tissue Cancer

As reviewed by Montelli et al. (2009), the potential clinical use of DC vaccines against brain tumors has also been investigated by some groups. The first DC vaccination study in patients with malignant glioma was reported in 2001 by [130], showing increased tumor-specific cytotoxicity in four out of seven patients treated with peptide-pulsed DCs. In a phase I clinical trial conducted by Sampson et al. [131], 13 patients with glioblastoma (GBM) and 3 with WHO grade III glioma were i.d. inoculated with autologous DC vaccine. Peripheral blood monocyte-derived DCs were pulsed with peptide from a mutated region of EGFRvIII conjugated with KLH (keyhole limpet hemocyanin). After three doses, immunization resulted in the restoration of immune responsiveness, which was followed only by grade I or II local reaction at the administration site. Treatment resulted in a median survival time of 110.8 weeks, which was higher than usually observed in patients under other types of therapy such as temozolomide (63.3 weeks [132]) and carmustine wafers (59.6 weeks [133]).

Parajuli et al. [134] studied *in vitro* the ability of different DC-vaccine strategies to induce T-cell response against malignant astrocytomas. Autologous monocyte-derived DCs were pulsed either with autologous tumor lysate, transfection with total tumor mRNA or by fusion of DCs with tumor cells. The authors concluded that all strategies used for pulsing DCs efficiently induced T-cell cytotoxicity, which was further improved by addition of CD40 ligand [135].

Twelve GBM patients followed in a phase I trial were treated with DC vaccines pulsed with peptides eluted from autologous tumor cells. After 3 doses, 50 % of the patients presented increased immunological response against autologous tumor cells and survival time was higher than historical control data [136].

In a very expressive clinical trial, 56 patients with relapsing GBM were treated with at least 3 doses of autologous DCs loaded with autologous tumor lysate, producing a 3-month median progression-free survival and a 9.6-month overall survival. Almost 15 % of patients presented a 2-year overall survival, although some of them have presented relapse during the follow-up [137]. In a phase II study, patients producing increased levels of IFN-γ showed higher overall survival than nonresponders [138].

Polarization of type 1 response can also be achieved by polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose (poly-ICLC), a type 1 IFN inducer. This product acts on TLR3 [139] to induce the production of IFN-γ, IL-6, TNF-γ, and chemokines including CCL2, CCL5, CCL20, and CXCL10 from astrocyte and microglia [140, 141]. Among the 38 patients with malignant glioma enrolled in the first clinical trial, those inoculated with poly-ICLC showed minimal toxicity associated with the treatment. Sixty-seven percent of the patients exhibited tumor regression or stabilization under radiological evaluation, with a 19-month median survival [142]. Antitumor response was associated with activation of 2′5′-oligoadenylate synthetases, which are antiviral proteins induced by type I IFN [143]. In another study, 30 adult patients with glioblastoma multiforme received poly-ICLC in combination with radiotherapy,

thereby demonstrating an advantage in relation to historical studies using radiotherapy alone [144]. Okada's group is currently analyzing the effect of associating poly-ICLC with DC vaccines generated under INF–α(αDC1), previously shown to be more effective than conventional DCs at inducing an antigen-specific CTL response [145].

19.8 Concluding Remarks

Despite their demonstrated effectiveness and promising results, the clinical use of DC vaccines is promising but not definitive. It can be partially explained by the difficulty of establishing a standard effective source of antigens and because several tumor-associated antigens are shared by normal cells. In addition, the increased Treg cells in advanced cancer, as well as other suppressor cells, can hinder the efficacy of a DC vaccine. In fact, even after activation, the autologous DCs of breast cancer patients induce higher levels of regulatory T cells (Treg) than DCs from healthy donors [146], which determines a low immunogenicity of autologous monocyte-derived DCs usually suppressed or induced to tolerance by Treg cytokines.

Reduction of Treg activity by blocking the regulatory molecule CTLA-4, through a monoclonal antibody, can be a good strategy to overcome this obstacle. The FDA reinforced this possibility through its 2011 approval of anti-CTLA-4 (ipilimumab – Yervoy; Bristol-Myers Squibb) for treatment of metastatic advanced melanoma. Treatment was well tolerated by patients and the combination with autologous DC vaccine or peptide-based vaccination was able to develop a significant antitumor response [147, 148].

In conclusion, despite these limitations, promising results are stimulating the search for the best pathways toward improving tumor immunogenicity, DCs' antigen-presenting function, responsiveness of effector cells in the tumor microenvironment, as well as overcoming the tolerogenic or suppressive status of the patient's immune system. Association of different immunotherapeutic approaches or combination of immunotherapy with chemotherapy [53] can open up new avenues for fighting cancer.

References

1. Langerhans P. Uber die nervender menschlichen haut. Virhouv Arch. 1868;44:13.
2. Prunieras M. Interactions between keratinocytes and dendritic cells. J Invest Dermatol. 1969;52(1):1–17.
3. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J Exp Med. 1973;137(5):1142–62.
4. Steinman RM, Adams JC, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. IV. Identification and distribution in mouse spleen. J Exp Med. 1975;141(4):804–20.
5. Steinman RM, Lustig DS, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. 3. Functional properties in vivo. J Exp Med. 1974;139(6):1431–45.
6. Steinman RM, Witmer MD. Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice. Proc Natl Acad Sci U S A. 1978;75(10):5132–6.
7. Steinman RM, Kaplan G, Witmer MD, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. V. Purification of spleen dendritic cells, new surface markers, and maintenance in vitro. J Exp Med. 1979;149(1):1–16.
8. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol. 2005;5(4):296–306.
9. Naik SH. Demystifying the development of dendritic cell subtypes, a little. Immunol Cell Biol. 2008; 86(5):439–52.
10. Satpathy AT, Wu X, Albring JC, Murphy KM. Re(de) fining the dendritic cell lineage. Nat Immunol. 2012;13(12):1145–54.
11. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. Annu Rev Immunol. 2000;18:767–811.
12. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;392(6673): 245–52.
13. Maazi H, Lam J, Lombardi V, Akbari O. Role of plasmacytoid dendritic cell subsets in allergic asthma. Allergy. 2013;68(6):695–701.
14. Trombetta ES, Mellman I. Cell biology of antigen processing in vitro and in vivo. Annu Rev Immunol. 2005;23:975–1028.
15. Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. J Exp Med. 1974;139(2):380–97.
16. Syme RM, Spurrell JC, Amankwah EK, Green FH, Mody CH. Primary dendritic cells phagocytose Cryptococcus neoformans via mannose receptors and Fcgamma receptor II for presentation to T lymphocytes. Infect Immun. 2002;70(11):5972–81.
17. Cambi A, Gijzen K, de Vries lJ, Torensma R, Joosten B, Adema GJ, et al. The C-type lectin DC-SIGN

(CD209) is an antigen-uptake receptor for Candida albicans on dendritic cells. Eur J Immunol. 2003;33(2):532–8.
18. Mahnke K, Guo M, Lee S, Sepulveda H, Swain SL, Nussenzweig M, et al. The dendritic cell receptor for endocytosis, DEC-205, can recycle and enhance antigen presentation via major histocompatibility complex class II-positive lysosomal compartments. J Cell Biol. 2000;151(3):673–84.
19. Steinman RM. The dendritic cell system and its role in immunogenicity. Annu Rev Immunol. 1991;9: 271–96.
20. Mohamadzadeh M, Mohamadzadeh H, Brammer M, Sestak K, Luftig RB. Identification of proteases employed by dendritic cells in the processing of protein purified derivative (PPD). J Immune Based Ther Vaccines. 2004;2(1):8.
21. Steinman RM. The control of immunity and tolerance by dendritic cell. Pathol Biol (Paris). 2003;51(2):59–60.
22. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. Annu Rev Immunol. 2003;21:685–711.
23. Sallusto F, Schaerli P, Loetscher P, Schaniel C, Lenig D, Mackay CR, et al. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. Eur J Immunol. 1998;28(9):2760–9.
24. Arina A, Tirapu I, Alfaro C, Rodriguez-Calvillo M, Mazzolini G, Inoges S, et al. Clinical implications of antigen transfer mechanisms from malignant to dendritic cells. exploiting cross-priming. Exp Hematol. 2002;30(12):1355–64.
25. Guermonprez P, Valladeau J, Zitvogel L, Thery C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. Annu Rev Immunol. 2002;20:621–67.
26. Yanagihara S, Komura E, Nagafune J, Watarai H, Yamaguchi Y. EBI1/CCR7 is a new member of dendritic cell chemokine receptor that is up-regulated upon maturation. J Immunol. 1998;161(6):3096–102.
27. Katou F, Ohtani H, Nakayama T, Ono K, Matsushima K, Saaristo A, et al. Macrophage-derived chemokine (MDC/CCL22) and CCR4 are involved in the formation of T lymphocyte-dendritic cell clusters in human inflamed skin and secondary lymphoid tissue. Am J Pathol. 2001;158(4):1263–70.
28. Adema GJ, Hartgers F, Verstraten R, de Vries E, Marland G, Menon S, et al. A dendritic-cell-derived C-C chemokine that preferentially attracts naive T cells. Nature. 1997;387(6634):713–7.
29. Malvicini M, Ingolotti M, Piccioni F, Garcia M, Bayo J, Atorrasagasti C, et al. Reversal of gastrointestinal carcinoma-induced immunosuppression and induction of antitumoural immunity by a combination of cyclophosphamide and gene transfer of IL-12. Mol Oncol. 2011;5(3):242–55.
30. Narumi K, Kondoh A, Udagawa T, Hara H, Goto N, Ikarashi Y, et al. Administration route-dependent induction of antitumor immunity by interferon-alpha gene transfer. Cancer Sci. 2010;101(7):1686–94.
31. Barbuto JA, Ensina LF, Neves AR, Bergami-Santos P, Leite KR, Marques R, et al. Dendritic cell-tumor cell hybrid vaccination for metastatic cancer. Cancer Immunol Immunother. 2004;53(12):1111–8.
32. Sallusto F, Lanzavecchia A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med. 1994;179(4):1109–18.
33. Wesa A, Kalinski P, Kirkwood JM, Tatsumi T, Storkus WJ. Polarized type-1 dendritic cells (DC1) producing high levels of IL-12 family members rescue patient TH1-type antimelanoma CD4+ T cell responses in vitro. J Immunother. 2007;30(1):75–82.
34. Giermasz AS, Urban JA, Nakamura Y, Watchmaker P, Cumberland RL, Gooding W, et al. Type-1 polarized dendritic cells primed for high IL-12 production show enhanced activity as cancer vaccines. Cancer Immunol Immunother. 2009;58(8):1329–36.
35. Marten A, Ziske C, Schottker B, Weineck S, Renoth S, Buttgereit P, et al. Transfection of dendritic cells (DCs) with the CIITA gene: increase in immunostimulatory activity of DCs. Cancer Gene Ther. 2001;8(3):211–9.
36. Henry F, Bretaudeau L, Hequet A, Barbieux I, Lieubeau B, Meflah K, et al. Role of antigen-presenting cells in long-term antitumor response based on tumor-derived apoptotic body vaccination. Pathobiology. 1999;67(5–6):306–10.
37. Gianotti L, Sargenti M, Galbiati F, Nespoli L, Brivio F, Rescigno M, et al. Phenotype and function of dendritic cells and T-lymphocyte polarization in the human colonic mucosa and adenocarcinoma. Eur J Surg Oncol. 2008;34(8):883–9.
38. Kalinski P, Schuitemaker JH, Hilkens CM, Wierenga EA, Kapsenberg ML. Final maturation of dendritic cells is associated with impaired responsiveness to IFN-gamma and to bacterial IL-12 inducers: decreased ability of mature dendritic cells to produce IL-12 during the interaction with Th cells. J Immunol. 1999;162(6):3231–6.
39. Kim KD, Choi SC, Kim A, Choe YK, Choe IS, Lim JS. Dendritic cell-tumor coculturing vaccine can induce antitumor immunity through both NK and CTL interaction. Int Immunopharmacol. 2001;1(12): 2117–29.
40. Chiyo M, Shimozato O, Iizasa T, Fujisawa T, Tagawa M. Antitumor effects produced by transduction of dendritic cells-derived heterodimeric cytokine genes in murine colon carcinoma cells. Anticancer Res. 2004;24(6):3763–7.
41. Jack AM, Aydin N, Montenegro G, Alam K, Wallack M. A novel dendritic cell-based cancer vaccine produces promising results in a syngenic CC-36 murine colon adenocarcinoma model. J Surg Res. 2007; 139(2):164–9.
42. Shan BE, Hao JS, Li QX, Tagawa M. Antitumor activity and immune enhancement of murine interleukin-23 expressed in murine colon carcinoma cells. Cell Mol Immunol. 2006;3(1):47–52.
43. Wurzenberger C, Koelzer VH, Schreiber S, Anz D, Vollmar AM, Schnurr M, et al. Short-term activation

induces multifunctional dendritic cells that generate potent antitumor T-cell responses in vivo. Cancer Immunol Immunother. 2009;58(6):901–13.
44. Luo Y, O'Hagan D, Zhou H, Singh M, Ulmer J, Reisfeld RA, et al. Plasmid DNA encoding human carcinoembryonic antigen (CEA) adsorbed onto cationic microparticles induces protective immunity against colon cancer in CEA-transgenic mice. Vaccine. 2003;21(17–18):1938–47.
45. Mackensen A, Herbst B, Chen JL, Kohler G, Noppen C, Herr W, et al. Phase I study in melanoma patients of a vaccine with peptide-pulsed dendritic cells generated in vitro from CD34(+) hematopoietic progenitor cells. Int J Cancer. 2000;86(3):385–92.
46. Okano F, Merad M, Furumoto K, Engleman EG. In vivo manipulation of dendritic cells overcomes tolerance to unmodified tumor-associated self antigens and induces potent antitumor immunity. J Immunol. 2005;174(5):2645–52.
47. Roux S, Apetoh L, Chalmin F, Ladoire S, Mignot G, Puig PE, et al. CD4+CD25+ Tregs control the TRAIL-dependent cytotoxicity of tumor-infiltrating DCs in rodent models of colon cancer. J Clin Invest. 2008;118(11):3751–61.
48. Marigo I, Dolcetti L, Serafini P, Zanovello P, Bronte V. Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. Immunol Rev. 2008;222:162–79.
49. Lu T, Ramakrishnan R, Altiok S, Youn JI, Cheng P, Celis E, et al. Tumor-infiltrating myeloid cells induce tumor cell resistance to cytotoxic T cells in mice. J Clin Invest. 2011;121(10):4015–29.
50. Youn JI, Gabrilovich DI. The biology of myeloid-derived suppressor cells: the blessing and the curse of morphological and functional heterogeneity. Eur J Immunol. 2010;40(11):2969–75.
51. Cheng P, Corzo CA, Luetteke N, Yu B, Nagaraj S, Bui MM, et al. Inhibition of dendritic cell differentiation and accumulation of myeloid-derived suppressor cells in cancer is regulated by S100A9 protein. J Exp Med. 2008;205(10):2235–49.
52. Hatfield P, Merrick AE, West E, O'Donnell D, Selby P, Vile R, et al. Optimization of dendritic cell loading with tumor cell lysates for cancer immunotherapy. J Immunother. 2008;31(7):620–32.
53. Kaneno R, Shurin GV, Kaneno FM, Naiditch H, Luo J, Shurin MR. Chemotherapeutic agents in low non-cytotoxic concentrations increase immunogenicity of human colon cancer cells. Cell Oncol (Dordr). 2011;34(2):97–106.
54. Dai S, Wan T, Wang B, Zhou X, Xiu F, Chen T, et al. More efficient induction of HLA-A*0201-restricted and carcinoembryonic antigen (CEA)-specific CTL response by immunization with exosomes prepared from heat-stressed CEA-positive tumor cells. Clin Cancer Res. 2005;11(20):7554–63.
55. Matera L, Forno S, Galetto A, Moro F, Garetto S, Mussa A. Increased expression of HSP70 by colon cancer cells is not always associated with access to the dendritic cell cross-presentation pathway. Cell Mol Biol Lett. 2007;12(2):268–79.
56. Qiu J, Li GW, Sui YF, Song HP, Si SY, Ge W. Heat-shocked tumor cell lysate-pulsed dendritic cells induce effective anti-tumor immune response in vivo. World J Gastroenterol. 2006;12(3):473–8.
57. Yasuda T, Kamigaki T, Nakamura T, Imanishi T, Hayashi S, Kawasaki K, et al. Dendritic cell-tumor cell hybrids enhance the induction of cytotoxic T lymphocytes against murine colon cancer: a comparative analysis of antigen loading methods for the vaccination of immunotherapeutic dendritic cells. Oncol Rep. 2006;16(6):1317–24.
58. Boczkowski D, Nair SK, Snyder D, Gilboa E. Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. J Exp Med. 1996;184(2):465–72.
59. Morse MA, Nair SK, Mosca PJ, Hobeika AC, Clay TM, Deng Y, et al. Immunotherapy with autologous, human dendritic cells transfected with carcinoembryonic antigen mRNA. Cancer Invest. 2003;21(3):341–9.
60. Strobel I, Berchtold S, Gotze A, Schulze U, Schuler G, Steinkasserer A. Human dendritic cells transfected with either RNA or DNA encoding influenza matrix protein M1 differ in their ability to stimulate cytotoxic T lymphocytes. Gene Ther. 2000;7(23):2028–35.
61. Mitchell DA, Nair SK. RNA-transfected dendritic cells in cancer immunotherapy. J Clin Invest. 2000;106(9):1065–9.
62. Van Tendeloo VF, Ponsaerts P, Lardon F, Nijs G, Lenjou M, Van Broeckhoven C, et al. Highly efficient gene delivery by mRNA electroporation in human hematopoietic cells: superiority to lipofection and passive pulsing of mRNA and to electroporation of plasmid cDNA for tumor antigen loading of dendritic cells. Blood. 2001;98(1):49–56.
63. Eppler E, Horig H, Kaufman HL, Groscurth P, Filgueira L. Carcinoembryonic antigen (CEA) presentation and specific T cell-priming by human dendritic cells transfected with CEA-mRNA. Eur J Cancer. 2002;38(1):184–93.
64. Ojima E, Inoue Y, Watanabe H, Hiro J, Toiyama Y, Miki C, et al. The optimal schedule for 5-fluorouracil radiosensitization in colon cancer cell lines. Oncol Rep. 2006;16(5):1085–91.
65. Bergant M, Meden L, Repnik U, Sojar V, Stanisavljevic D, Jeras M. Preparation of native and amplified tumour RNA for dendritic cell transfection and generation of in vitro anti-tumour CTL responses. Immunobiology. 2006;211(3):179–89.
66. Nair SK, Boczkowski D, Morse M, Cumming RI, Lyerly HK, Gilboa E. Induction of primary carcinoembryonic antigen (CEA)-specific cytotoxic T lymphocytes in vitro using human dendritic cells transfected with RNA. Nat Biotechnol. 1998;16(4):364–9.
67. Sakakibara M, Kanto T, Hayakawa M, Kuroda S, Miyatake H, Itose I, et al. Comprehensive immunological analyses of colorectal cancer patients in the phase I/II study of quickly matured dendritic cell vaccine pulsed with carcinoembryonic antigen peptide. Cancer Immunol Immunother. 2011;60(11):1565–75.

68. Dall'Oglio M, Srougi M, Barbuto JA. Complete response of metastatic renal cancer with dendritic cell vaccine. Int Braz J Urol. 2003;29(6):517–9.
69. Xu F, Ye YJ, Cui ZR, Wang S. Allogeneic dendritomas induce anti-tumour immunity against metastatic colon cancer. Scand J Immunol. 2005;61(4):364–9.
70. Yasuda T, Kamigaki T, Kawasaki K, Nakamura T, Yamamoto M, Kanemitsu K, et al. Superior antitumor protection and therapeutic efficacy of vaccination with allogeneic and semiallogeneic dendritic cell/tumor cell fusion hybrids for murine colon adenocarcinoma. Cancer Immunol Immunother. 2007;56(7):1025–36.
71. Chaput N, Taieb J, Schartz NE, Andre F, Angevin E, Zitvogel L. Exosome-based immunotherapy. Cancer Immunol Immunother. 2004;53(3):234–9.
72. Chaput N, Schartz NE, Andre F, Taieb J, Novault S, Bonnaventure P, et al. Exosomes as potent cell-free peptide-based vaccine. II. Exosomes in CpG adjuvants efficiently prime naive Tc1 lymphocytes leading to tumor rejection. J Immunol. 2004;172(4):2137–46.
73. Viaud S, Ullrich E, Zitvogel L, Chaput N. Exosomes for the treatment of human malignancies. Horm Metab Res. 2008;40(2):82–8.
74. Mignot G, Roux S, Thery C, Segura E, Zitvogel L. Prospects for exosomes in immunotherapy of cancer. J Cell Mol Med. 2006;10(2):376–88.
75. Andre F, Schartz NE, Chaput N, Flament C, Raposo G, Amigorena S, et al. Tumor-derived exosomes: a new source of tumor rejection antigens. Vaccine. 2002;20 Suppl 4:A28–31.
76. Andre F, Chaput N, Schartz NE, Flament C, Aubert N, Bernard J, et al. Exosomes as potent cell-free peptide-based vaccine. I. Dendritic cell-derived exosomes transfer functional MHC class I/peptide complexes to dendritic cells. J Immunol. 2004;172(4):2126–36.
77. Viaud S, Thery C, Ploix S, Tursz T, Lapierre V, Lantz O, et al. Dendritic cell-derived exosomes for cancer immunotherapy: what's next? Cancer Res. 2010;70(4):1281–5.
78. Romagnoli GG, Toniolo PA, Migliori IK, Caldini EG, Ferreira MA, Pizzo CR, et al. Tumor cells incorporate exosomes derived from dendritic cells through a mechanism involving the tetraspanin CD9. Exosomes Microvesicles. 2013;4:12.
79. Li Z, Menoret A, Srivastava P. Roles of heat-shock proteins in antigen presentation and cross-presentation. Curr Opin Immunol. 2002;14(1):45–51.
80. Srivastava P. Roles of heat-shock proteins in innate and adaptive immunity. Nat Rev Immunol. 2002;2(3):185–94.
81. Tamura Y, Peng P, Liu K, Daou M, Srivastava PK. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science. 1997;278(5335):117–20.
82. Castellino F, Boucher PE, Eichelberg K, Mayhew M, Rothman JE, Houghton AN, et al. Receptor-mediated uptake of antigen/heat shock protein complexes results in major histocompatibility complex class I antigen presentation via two distinct processing pathways. J Exp Med. 2000;191(11):1957–64.
83. Singh-Jasuja H, Scherer HU, Hilf N, Arnold-Schild D, Rammensee HG, Toes RE, et al. The heat shock protein gp96 induces maturation of dendritic cells and down-regulation of its receptor. Eur J Immunol. 2000;30(8):2211–5.
84. Blachere NE, Li Z, Chandawarkar RY, Suto R, Jaikaria NS, Basu S, et al. Heat shock protein-peptide complexes, reconstituted in vitro, elicit peptide-specific cytotoxic T lymphocyte response and tumor immunity. J Exp Med. 1997;186(8):1315–22.
85. Kurotaki T, Tamura Y, Ueda G, Oura J, Kutomi G, Hirohashi Y, et al. Efficient cross-presentation by heat shock protein 90-peptide complex-loaded dendritic cells via an endosomal pathway. J Immunol. 2007;179(3):1803–13.
86. Binder RJ, Anderson KM, Basu S, Srivastava PK. Cutting edge: heat shock protein gp96 induces maturation and migration of CD11c+cells in vivo. J Immunol. 2000;165(11):6029–35.
87. Binder RJ, Han DK, Srivastava PK. CD91: a receptor for heat shock protein gp96. Nat Immunol. 2000;1(2):151–5.
88. Chandawarkar RY, Wagh MS, Srivastava PK. The dual nature of specific immunological activity of tumor-derived gp96 preparations. J Exp Med. 1999;189(9):1437–42.
89. Wang HH, Mao CY, Teng LS, Cao J. Recent advances in heat shock protein-based cancer vaccines. Hepatobiliary Pancreat Dis. 2006;5(1):22–7.
90. Prince HM, Wall DM, Ritchie D, Honemann D, Harrrison S, Quach H, et al. In vivo tracking of dendritic cells in patients with multiple myeloma. J Immunother. 2008;31(2):166–79.
91. Morse MA, Coleman RE, Akabani G, Niehaus N, Coleman D, Lyerly HK. Migration of human dendritic cells after injection in patients with metastatic malignancies. Cancer Res. 1999;59(1):56–8.
92. Huck SP, Tang SC, Andrew KA, Yang J, Harper JL, Ronchese F. Activation and route of administration both determine the ability of bone marrow-derived dendritic cells to accumulate in secondary lymphoid organs and prime CD8+ T cells against tumors. Cancer Immunol Immunother. 2008;57(1):63–71.
93. Verdijk P, Aarntzen EH, Punt CJ, de Vries IJ, Figdor CG. Maximizing dendritic cell migration in cancer immunotherapy. Expert Opin Biol Ther. 2008;8(7):865–74.
94. Eggert AA, Schreurs MW, Boerman OC, Oyen WJ, de Boer AJ, Punt CJ, et al. Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration. Cancer Res. 1999;59(14):3340–5.
95. Mullins DW, Sheasley SL, Ream RM, Bullock TN, Fu YX, Engelhard VH. Route of immunization with peptide-pulsed dendritic cells controls the distribution of memory and effector T cells in lymphoid tissues and determines the pattern of regional tumor control. J Exp Med. 2003;198(7):1023–34.

96. Okada N, Mori N, Koretomo R, Okada Y, Nakayama T, Yoshie O, et al. Augmentation of the migratory ability of DC-based vaccine into regional lymph nodes by efficient CCR7 gene transduction. Gene Ther. 2005;12(2):129–39.
97. Feijoo E, Alfaro C, Mazzolini G, Serra P, Penuelas I, Arina A, et al. Dendritic cells delivered inside human carcinomas are sequestered by interleukin-8. Int J Cancer. 2005;116(2):275–81.
98. Mazzolini G, Alfaro C, Sangro B, Feijoo E, Ruiz J, Benito A, et al. Intratumoral injection of dendritic cells engineered to secrete interleukin-12 by recombinant adenovirus in patients with metastatic gastrointestinal carcinomas. J Clin Oncol. 2005;23(5): 999–1010.
99. Guo J, Zhu J, Sheng X, Wang X, Qu L, Han Y, et al. Intratumoral injection of dendritic cells in combination with local hyperthermia induces systemic antitumor effect in patients with advanced melanoma. Int J Cancer. 2007;120(11):2418–25.
100. Mirvish ED, Pomerantz RG, Falo LD, Geskin LJ. Dendritic cell vaccines in cancer: obstacles to overcome. In: Shurin MR, Salter RD, editors. Dendritic cells in cancer. New York: Springer; 2009. p. 309–30.
101. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA. 2011; 61(2):69–90.
102. Roehl KA, Han M, Ramos CG, Antenor JA, Catalona WJ. Cancer progression and survival rates following anatomical radical retropubic prostatectomy in 3,478 consecutive patients: long-term results. J Urol. 2004;172(3):910–4.
103. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411–22.
104. Higano CS, Small EJ, Schellhammer P, Yasothan U, Gubernick S, Kirkpatrick P, et al. Sipuleucel-T. Nat Rev Drug Discov. 2009;9(7):513–4.
105. Cheever MA, Higano CS. PROVENGE (Sipuleucel-T) in prostate cancer: the first FDA-approved therapeutic cancer vaccine. Clin Cancer Res. 2011;17(11):3520–6.
106. Di Lorenzo G, Ferro M, Buonerba C. Sipuleucel-T (Provenge(R)) for castration-resistant prostate cancer. BJU Int. 2012;110(2 Pt 2):E99–104.
107. Quintero IB, Araujo CL, Pulkka AE, Wirkkala RS, Herrala AM, Eskelinen EL, et al. Prostatic acid phosphatase is not a prostate specific target. Cancer Res. 2007;67(14):6549–54.
108. Freedland SJ, Mangold LA, Walsh PC, Partin AW. The prostatic specific antigen era is alive and well: prostatic specific antigen and biochemical progression following radical prostatectomy. J Urol. 2005;174(4 Pt 1):1276–81; discussion 1281; author reply 1281.
109. Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol. 2006;24(19):3089–94.
110. Barrou B, Benoit G, Ouldkaci M, Cussenot O, Salcedo M, Agrawal S, et al. Vaccination of prostatectomized prostate cancer patients in biochemical relapse, with autologous dendritic cells pulsed with recombinant human PSA. Cancer Immunol Immunother. 2004;53(5):453–60.
111. Fong L, Brockstedt D, Benike C, Breen JK, Strang G, Ruegg CL, et al. Dendritic cell-based xenoantigen vaccination for prostate cancer immunotherapy. J Immunol. 2001;167(12):7150–6.
112. Lundqvist A, Palmborg A, Bidla G, Whelan M, Pandha H, Pisa P. Allogeneic tumor-dendritic cell fusion vaccines for generation of broad prostate cancer T-cell responses. Med Oncol. 2004;21(2): 155–65.
113. Pandha HS, John RJ, Hutchinson J, James N, Whelan M, Corbishley C, et al. Dendritic cell immunotherapy for urological cancers using cryopreserved allogeneic tumour lysate-pulsed cells: a phase I/II study. BJU Int. 2004;94(3):412–8.
114. Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, et al. Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med. 1998;4(3):328–32.
115. Salcedo M, Bercovici N, Taylor R, Vereecken P, Massicard S, Duriau D, et al. Vaccination of melanoma patients using dendritic cells loaded with an allogeneic tumor cell lysate. Cancer Immunol Immunother. 2006;55(7):819–29.
116. Ellebaek E, Engell-Noerregaard L, Iversen TZ, Froesig TM, Munir S, Hadrup SR, et al. Metastatic melanoma patients treated with dendritic cell vaccination, Interleukin-2 and metronomic cyclophosphamide: results from a phase II trial. Cancer Immunol Immunother. 2012;61(10):1791–804.
117. Kyte JA, Kvalheim G, Aamdal S, Saeboe-Larssen S, Gaudernack G. Preclinical full-scale evaluation of dendritic cells transfected with autologous tumor-mRNA for melanoma vaccination. Cancer Gene Ther. 2005;12(6):579–91.
118. Kyte JA, Gaudernack G. Immuno-gene therapy of cancer with tumour-mRNA transfected dendritic cells. Cancer Immunol Immunother. 2006;55(11):1432–42.
119. das Neves FJ, Mattos IE, Koifman RJ. Colon and rectal cancer mortality in Brazilian capitals, 1980–1997. Arq Gastroenterol. 2005;42(1):63–70.
120. Pavli P, Hume DA, Van De Pol E, Doe WF. Dendritic cells, the major antigen presenting cells of the human colonic lamina propria. Immunology. 1993;78(1): 132–41.
121. Klaas M. Security, stat! ED safety requires cooperation between departments. Health Facil Manag. 2005;18(1):22–6.
122. Nonaka M, Ma BY, Murai R, Nakamura N, Baba M, Kawasaki N, et al. Glycosylation-dependent interactions of C-type lectin DC-SIGN with colorectal tumor-associated Lewis glycans impair the function

and differentiation of monocyte-derived dendritic cells. J Immunol. 2008;180(5):3347–56.
123. Schwaab T, Weiss JE, Schned AR, Barth Jr RJ. Dendritic cell infiltration in colon cancer. J Immunother. 2001;24(2):130–7.
124. Chaux P, Moutet M, Faivre J, Martin F, Martin M. Inflammatory cells infiltrating human colorectal carcinomas express HLA class II but not B7-1 and B7-2 costimulatory molecules of the T-cell activation. Lab Invest. 1996;74(5):975–83.
125. Chaux P, Favre N, Martin M, Martin F. Tumor-infiltrating dendritic cells are defective in their antigen-presenting function and inducible B7 expression in rats. Int J Cancer. 1997;72(4):619–24.
126. Kim SG, Park MY, Kim CH, Sohn HJ, Kim HS, Park JS, et al. Modification of CEA with both CRT and TAT PTD induces potent anti-tumor immune responses in RNA-pulsed DC vaccination. Vaccine. 2008;26(50):6433–40.
127. Nencioni A, Muller MR, Grunebach F, Garuti A, Mingari MC, Patrone F, et al. Dendritic cells transfected with tumor RNA for the induction of antitumor CTL in colorectal cancer. Cancer Gene Ther. 2003;10(3):209–14.
128. Nair SK, Morse M, Boczkowski D, Cumming RI, Vasovic L, Gilboa E, et al. Induction of tumor-specific cytotoxic T lymphocytes in cancer patients by autologous tumor RNA-transfected dendritic cells. Ann Surg. 2002;235(4):540–9.
129. Yamaguchi S, Tatsumi T, Takehara T, Sakamori R, Uemura A, Mizushima T, et al. Immunotherapy of murine colon cancer using receptor tyrosine kinase EphA2-derived peptide-pulsed dendritic cell vaccines. Cancer. 2007;110(7):1469–77.
130. Yu JS, Wheeler CJ, Zeltzer PM, Ying H, Finger DN, Lee PK, et al. Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. Cancer Res. 2001;61(3):842–7.
131. Sampson JH, Archer GE, Mitchell DA, Heimberger AB, Bigner DD. Tumor-specific immunotherapy targeting the EGFRvIII mutation in patients with malignant glioma. Semin Immunol. 2008;20(5): 267–75.
132. Stupp R, van den Bent MJ, Hegi ME. Optimal role of temozolomide in the treatment of malignant gliomas. Curr Neurol Neurosci Rep. 2005;5(3):198–206.
133. Westphal M, Hilt DC, Bortey E, Delavault P, Olivares R, Warnke PC, et al. A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant glioma. Neuro Oncol. 2003;5(2):79–88.
134. Parajuli P, Mathupala S, Sloan AE. Systematic comparison of dendritic cell-based immunotherapeutic strategies for malignant gliomas: in vitro induction of cytolytic and natural killer-like T cells. Neurosurgery. 2004;55(5):1194–204.
135. Sloan AE, Parajuli, P, Mathupala, S. DC-tumor cell fusion for induction of tumor-specific T-cell response against malignant brain tumors: comparison with DC pulsed with total tumor RNA or tumor lysate. Proceeding of American Association for Cancer Research, San Francisco, CA. 2002.
136. Liau LM, Prins RM, Kiertscher SM, Odesa SK, Kremen TJ, Giovannone AJ, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. Clin Cancer Res. 2005;11(15):5515–25.
137. De Vleeschouwer S, Fieuws S, Rutkowski S, Van Calenbergh F, Van Loon J, Goffin J, et al. Postoperative adjuvant dendritic cell-based immunotherapy in patients with relapsed glioblastoma multiforme. Clin Cancer Res. 2008;14(10):3098–104.
138. Wheeler CJ, Black KL, Liu G, Mazer M, Zhang XX, Pepkowitz S, et al. Vaccination elicits correlated immune and clinical responses in glioblastoma multiforme patients. Cancer Res. 2008;68(14): 5955–64.
139. Trumpfheller C, Caskey M, Nchinda G, Longhi MP, Mizenina O, Huang Y, et al. The microbial mimic poly IC induces durable and protective CD4+ T cell immunity together with a dendritic cell targeted vaccine. Proc Natl Acad Sci U S A. 2008;105(7): 2574–9.
140. Farina C, Krumbholz M, Giese T, Hartmann G, Aloisi F, Meinl E. Preferential expression and function of Toll-like receptor 3 in human astrocytes. J Neuroimmunol. 2005;159(1–2):12–9.
141. Park C, Lee S, Cho IH, Lee HK, Kim D, Choi SY, et al. TLR3-mediated signal induces proinflammatory cytokine and chemokine gene expression in astrocytes: differential signaling mechanisms of TLR3-induced IP-10 and IL-8 gene expression. Glia. 2006;53(3):248–56.
142. Salazar AM, Levy HB, Ondra S, Kende M, Scherokman B, Brown D, et al. Long-term treatment of malignant gliomas with intramuscularly administered polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethylcellulose: an open pilot study. Neurosurgery. 1996;38(6):1096–103; discussion 1103–4.
143. Takahashi A, Iwasaki Y, Miyaike J, Taniguchi H, Shimomura H, Hanafusa T, et al. Quantitative analysis of p40/p46 and p69/p71 forms of 2′,5′-oligoadenylate synthetase mRNA by competitive PCR and its clinical application. Clin Chem. 2002;48(9):1551–9.
144. Butowski N, Chang SM, Junck L, DeAngelis LM, Abrey L, Fink K, et al. A phase II clinical trial of poly-ICLC with radiation for adult patients with newly diagnosed supratentorial glioblastoma: a North American Brain Tumor Consortium (NABTC01-05). J Neuro-Oncol. 2009;91(2):175–82.
145. Mailliard RB, Wankowicz-Kalinska A, Cai Q, Wesa A, Hilkens CM, Kapsenberg ML, et al. Alpha-type-1 polarized dendritic cells: a novel immunization tool with optimized CTL-inducing activity. Cancer Res. 2004;64(17):5934–7.

146. Ramos RN, Chin LS, Dos Santos AP, Bergami-Santos PC, Laginha F, Barbuto JA. Monocyte-derived dendritic cells from breast cancer patients are biased to induce CD4+CD25+Foxp3+ regulatory T cells. J Leukoc Biol. 2012;92(3):673–82.
147. Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci U S A. 2003;100(14):8372–7.
148. Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci U S A. 2003;100(8):4712–7.
149. Shedlock DJ, Shen H. Requirement for CD4 T cell help in generating functional CD8 T cell memory. Science 2003;300(5617):337–9.
150. Janssen EM, Lemmens EE, Wolfe T, Christen U, von Herrath MG, Schoenberger SP. CD4+ T cells are required for secondary expansion and memory in CD8+ T lymphocytes. Nature 2003;421(6925):852–6.
151. Montelli T de C, Peraçoli MT, Rogatto SR, Kaneno R, do Prado CH, Rocha P de M. Genetic and modifying factors that determine the risk of brain tumors. Cent Nerv Syst Agents Med Chem. 2011;11(1):8–30.