Review

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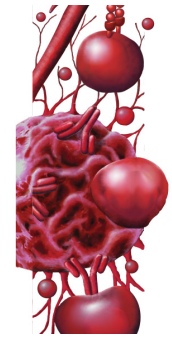
Immunotherapy



Dendritic cell-based vaccines: clinical applications in breast cancer

Recent evidence suggests that the immune system is involved in the carcinogenesis process and the antitumor immune responses impact the clinical outcome, thus emphasizing the concept of cancer immune surveillance. In this context, dendritic cells (DCs) seem to play a crucial role, as they are the most potent APCs and are able to stimulate naive T lymphocytes and to generate memory T lymphocytes. Immunotherapy with DC-based vaccines is a very attractive approach to treat cancer, offering the potential for high tumor-specific cytotoxicity. Although breast cancer (BC) is traditionally considered a poorly immunogenic tumor, increasing numbers of both preclinical and clinical studies demonstrate that vaccination with DCs is capable of inducing an antitumor-specific response, while being well tolerated and safe. However, clinical objective responses are still disappointing and many reasons may explain the difficulty of developing effective DC-based therapies for BC. In this review, we discuss the characteristics of DCs, and the major clinical indications for DC-based immunotherapy in BC with related drawbacks.

Keywords: breast cancer • dendritic cells • immune response • immunotherapies • vaccines

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The immune system plays a critical role in the development of cancer. Both the innate and acquired immune systems are able to identify transformed cancer cells – recognized as nonself – thus generating a specific immune response. The aim of the immune response is to destroy the transformed cells in order to prevent their proliferation and, consequently, tumor growth [1].

Immunotherapy is an emerging and increasingly promising approach to treat cancer [2]. Several efforts have been made in the recent years to identify molecules involved in the immune response to develop potential immune targets to treat cancers [3]. Breast cancer (BC) is the most common type of cancer among women. Despite the huge improvement in BC outcome with current multimodality approaches, approximately 20–30% of BC patients still relapse, even many years after diagnosis [4]. In contrast to melanoma and renal cell carcinoma (RCC), which have been considered more responsive to immunotherapies, BC has been traditionally considered poorly immunogenic, as it does not occur at higher incidence in the immunosuppressed populations who have been treated with immunosuppressive therapies [5]. Nonetheless, despite poor influence on primary tumor growth, the immune system seems to be effective in preventing BC metastases [6–8]. Different reasons can explain this limit, such as the heterogeneous expression of tumor antigens within the primary tumor or its metastases, the modification of antigenic profile during the tumor progress, and the low levels of the antigen, MHC proteins and other costimulatory proteins necessary to generate a strong immune response. On the other hand, the tumor microenvironment releases immune-suppressive factors that make antigen presentation difficult, with a negative impact on the immune response [9]. However, as recently exemplified in metastatic non-small-cell lung cancer, even tumor types traditionally not considered to be responsive

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to immunotherapy could be immunogenic with appropriate immune activation [10]. Thus, immunotherapy is now widely recognized as a key element in the treatment of cancer, including BC [11]. As dendritic cells (DCs) are considered the strongest stimulators of T-cell responses and play a crucial role in the initiation of primary immune response, different studies have exploited the potential effectiveness of DC-based vaccines in BC [12].

Immune system & cancer

Active immunotherapy in BC enables the immune system to discover neoplastic growth and to avoid carcinogenesis and reject transformed cells. Immune response can lead to the rejection of cancer, but can also have regulatory effects that promote tumor growth (i.e., immunoediting) [13]. Different mechanisms are involved in immune evasion, such as the defects in antigen presentation, the downregulation of adhesion molecules, the production of immunosuppressive factors and molecules, and the induction of mechanisms of immune tolerance (Box 1 & Figure 1) [3,14]. Accordingly, cancer develops due to selection of less-immunogenic tumor cells (immunoediting) and increased effectiveness of tumor-mediated immunosuppression (immune subversion) [13].

Emerging data also suggest that the killing of cancer cells by the immune system depends on the type of immune response elicited. A tumor-directed immune response involving $CD8^+$ cytotoxic T lymphocytes (CTLs), Th1 cells and NK cells seems to prevent tumor development and progression; conversely, the immune response that involves B cells and activation of humoral immunity and/or a Th2 response can encourage tumor growth and progression [15]. So, the induction of CTLs directed against tumor antigens *in vivo* is the attractive effect of a specific immunotherapy, considering that these immune cells are mostly responsible for tumor elimination [16]. The aim is to develop a specific and long-lasting immune response able to eliminate cancer cells without harming normal tissue.

In recent years, the knowledge of the potential control of a tumor by the immune system has allowed to test the efficacy of antitumor immunization strategies. In particular, the use of immunogenic APCs, such as DCs loaded with tumor antigens is now considered one of the most promising approaches in cancer immunotherapy, due to their notable ability to stimulate naive T lymphocytes and generate memory T lymphocytes.

DC biology & DC-based vaccines

DCs are a heterogeneous population of leukocytes that work as the most effective APCs, acting as messengers between the innate and adaptive immunity [17]. Immature DCs (iDCs) arising from bone marrow precursors are characterized by high endocytic activity and low T-cell activation, and they probably encourage antigen-specific tolerance rather than immunity [18]. Once iDCs get in contact with an antigen, they are activated into mature DCs by pathogen-associated molecular patterns (PAMPs) via Toll-like receptors (TLRs), inflammatory cytokines and prostaglandins released in the environment [19]. These mature DCs expressing high levels of costimulatory and MHC molecules (CD80 and CD86) migrate to lymphoid organs where they activate T cells through interactions between CD40 (expressed by DCs) and CD40 ligand (expressed by the T cells), thus generating an antigen-specific response to kill antigens (Figure 1) [20].

Many studies revealed that in cancer patients, DCs present abnormalities that make T-cell activation against tumors difficult, because of reduced uptake and processing of antigens, low expression of costimulatory signals, ineffective motility and migration towards specific chemokines, and decreased production of IL-12 and so on [21].

Nonproductive interactions between T cells and DCs on margins of murine breast tumors have been shown [22]. In this model, tumor-infiltrating DCs presented tumor-associated antigens (TAAs) to T cells and created stable interactions with infiltrating tumor-specific T cells, but this interaction appears to be insufficient to sustain CTL activity. Defects in DC maturation and migration from periphery to lymphoid organs, where they activate immune response, may complicate the development of an immune response [23,24]. Moreover, patients with operable BC have been shown to have peripheral and lymph nodal DCs less able to stimulate leukocytes, with low expression of HLA-DR and CD86, and with low ability to induce IL-12 secretion *in vitro* as compared with healthy donors [25].

In order to overcome these problems and to improve the immune function of these cells, it would be better to utilize DCs taken from the patient and manipulated *ex vivo*. A large number of cells with the DC phenotype and functional properties can be generated from bone marrow precursors ($CD34^+$) or peripheral blood monocytes in the presence of a cocktail of cytokines including GM-CSF and IL-4 [26]. These APCs, efficient in antigen uptake but with limited capacity to stimulate T-cell proliferation and to induce antigen-specific CTLs, are subsequently stimulated by exposure to activating factors (i.e., TNF-α, TLR ligands, CD40 ligand and monocyte-conditioned medium) or other types of cytokines to increase their immune-stimulatory capacity [27].

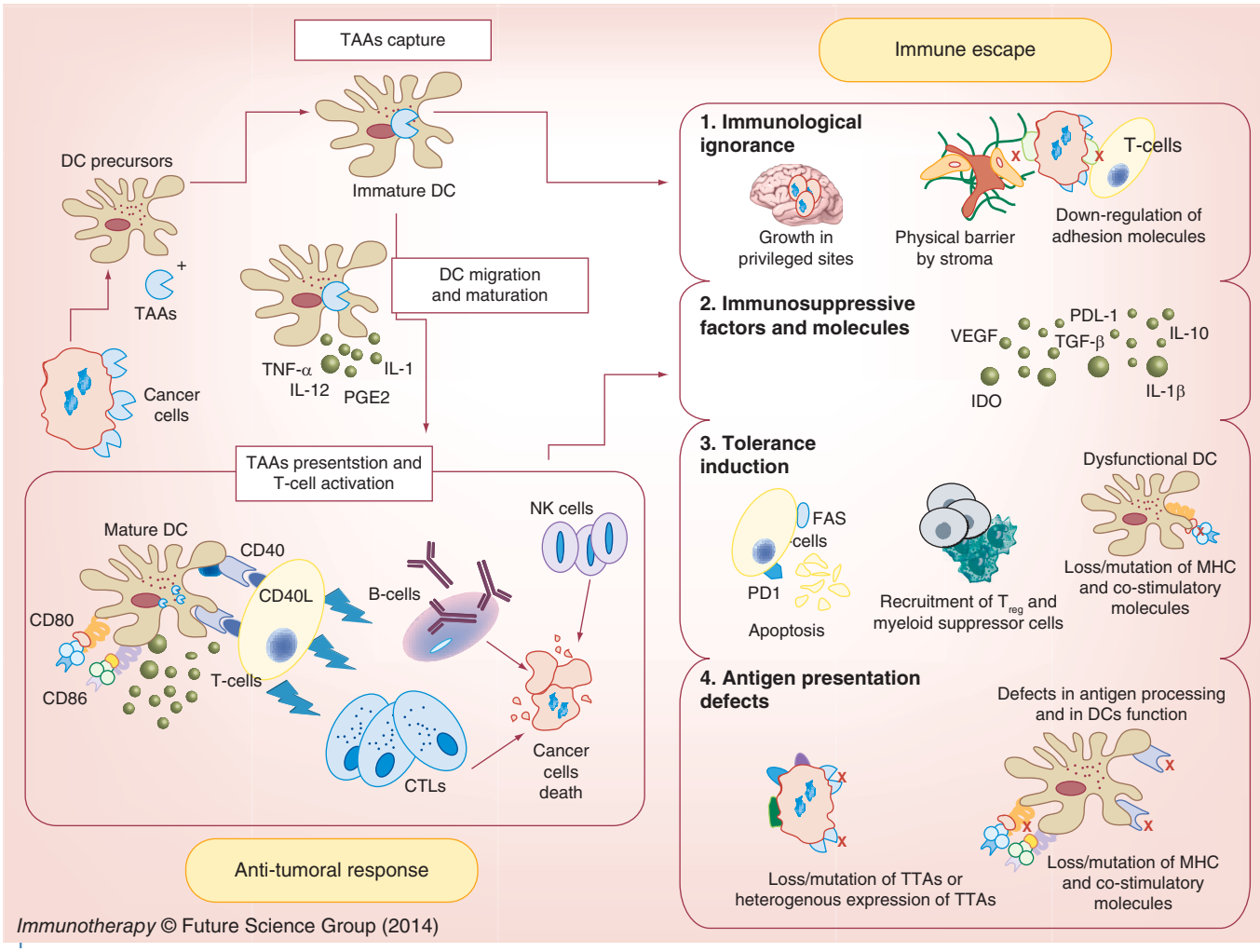

Figure 1. Immature dendritic cells derived from bone marrow precursors. Upon encounter with TAAs, immature DCs are induced to mature by inflammatory cytokines and prostaglandins relased in microenvironment. These mature DCs migrate in lymphoid organs where they interact with $CD8^+$ and $CD4^+$ T lymphocytes. $CD8^+$ cells, once activated, become CTLs that will be mostly responsible for the eradication of malignant cells. Moreover, in many cases cancer cells can avoid immune response and allow the tumor growth. Different mechanisms such as the immunological ignorance, the production of immunosuppressive factors and molecules, the induction of immune tolerance and defects in antigen presentation are involved in immune evasion.
CTL: Cytotoxic T lymphocyte; DC: Dendritic cell; TAA: Tumor-associated antigen.

After isolation and maturation, DCs are loaded with tumor antigens, administered in different forms (DNA, RNA, proteins, peptides, viruses or cell lyzates) for further presentation to T lymphocytes. Thus, the use of *ex vivo*-generated DCs is profitable, because it allows to overcome the difficulties resulting from their compromised immunological function to develop an adequate immune response against the tumor.

Preclinical & clinical studies with DC-based vaccines

Melanoma is the cancer type most frequently treated with DC-based vaccinations, followed by prostate cancer, RCC, BC, multiple myeloma, leukemia, colorectal cancer and glioma [28]. Particularly, melanoma and RCC have traditionally been considered more responsive to immunotherapies, due to their high immunogenicity. Immunotherapy is a cornerstone in the treatment of melanoma. Besides the demonstrated survival benefit achieved with an inhibitor of the gene coding RAF (*B-RAF;* vemurafenib) and a CTLA-4 antibody (ipilimumab) [29], DC-based vaccines have been demonstrated to be safe and effective in treating metastatic patients [30].

Similar to melanoma, RCC and prostate cancer have also continued to be the subject of further DC vaccination studies. After unsatisfactory results with immune-directed agents such as IL-2 or IFN-α, metastatic RCC patients' outcome has markedly improved with the introduction of novel agents targeting angiogenesis and signal transduction pathways (i.e., VEGF tyrosine kinase inhibitor [TKI] and mTOR inhibitor) [31]. Still,

Box 1. Mechanisms of tumor escape from the immune system.

Immunological ignorance
- Lack of danger signals
- Growth in immune privileged sites (i.e., brain)
- Downregulation of adhesion molecules and improper interaction between the immune system and tumor cells
- Physical barrier by stroma

Antigen presentation defects
- Loss or mutation of tumor antigens
- Heterogeneous expression of antigens
- Loss or mutation of MHC molecules
- Chronic antigen stimulation
- Defects in antigen processing (e.g., defects in transporter associated with antigen processing or proteasome subunits or tapasin)
- Defect of antigen-presenting cell functions

Immunosuppressive factors & molecules
- Inhibitory cytokines (e.g., TGF-β, IL-10, VEGF, IL-6 and IL-1β)
- Inibitory prostaglandins (i.e., PGE_2)
- Inhibitory signaling molecules (i.e., PD-1 ligands)
- Inhibitory enzyme (production of IDO from dendritic cells)

Tolerance induction
- Lack of costimulatory molecules
- Induction of T-cell apoptosis via PD-1 and Fas
- Recruitment of myeloid suppressor cells
- Induction of T-cell anergy
- Dysfunctional dendritic cells
- Generation of Tregs
- Immune deviation (Th2 response vs Th1 response)

newer immune approaches include anti-CTLA-4 [32] and anti-PD1 agents [9], and DC vaccines have been investigated for the treatment of metastatic RCC with encouraging results [33].

Moreover, autologous DC vaccines have recently established a role in prostate cancer therapy; indeed, the US FDA has approved the first cancer vaccine, sipuleucel-T, for asymptomatic or minimally symptomatic castration-resistant prostate cancer [34].

Recently, the better knowledge of BC biology has allowed the emergence of some immunotherapeutic strategies, despite this disease not traditionally being perceived as an immunogenic tumor. The use of DCs for cancer immunotherapy provides an interesting opportunity to overcome the relative nonimmunogenicity of BC and despite the limited success of such an approach, several preclinical and clinical studies have been conducted.

Preclinical studies in BC

Initial studies indicated that BC-infiltrating DCs were detected in >40% of patients with early and advanced BC, despite no correlation with outcome being observed [35]. DCs in BC seem to be able to provide a memory response to tumor antigens and to inhibit the tumor growth [36,37]. Gong *et al.* demonstrated that fusion of DCs with BC cells elicited autologous CTLs able to lyze cancer cells [38]. Furthermore, DCs loaded with allogeneic BC cells stimulated tumor-reactive CTLs with consequent destruction of target cells [39]. To improve immunogenicity of human EGFR2 (HER2), HER2-positive BC mice were immunized with DCs expressing the DEC205 receptor and high levels of T- and B-cell immunity were observed, despite the low amount of HER2 protein [40]. Recently, some researchers have investigated the capacity to overcome resistance to trastuzumab (an antibody to HER2), using an OVA-specific DC-released exosome (EXOOVA)-targeted $CD4^+$ T cell-based (OVA-TEXO) vaccine against neu-expressing Tg1-1 BC in the transgenic FVBneuN mice, resulting in the development of protective immunity [41]. The use of genetically modified DCs was also evaluated though HER2 adenovirus-transduced DCs, which prevented the growth of BC in HER2-transgenic mice [42]. The effectiveness of a whole-cell BC vaccine in mice was evaluated using an immunocytokine composed of IL-2 with an antibody directed to an immune-suppressive factor, phosphatidylserine. A total of 80% of mice survived free of tumor and their splenocytes had significantly higher specific cytotoxicity than splenocytes from control mice [43]. This study demonstrates the importance of immune-modulatory factors in development of an adequate immune response. In the preclinical setting combination therapy was evaluated as well. Zheng *et al.*

Table 1. Clinical trials for dendritic cell vaccine-based therapies.

NCT number	Study Phase	Estimated enrollment (n)†	Primary end point	Setting	Type of therapy (intervention)	Status
NCT01730118	I	65	Safety/toxicity/ immunogenity	Metastatic	Autologous adenovirus HER2-transduced DC vaccine	Ongoing
NCT0088985	II	55	Response rate	Locally recurrent or metastatic	Autologous DCs pulsed with E75 and E90 peptides with trastuzumab and vinorelbine	Completed
NCT01042535	I/II	37	MTD/safety	Metastatic	Adenovirus p53-transduced DCs with 1-methyl-D-tryptophan	Ongoing
NCT00266110	II	26	Efficacy	Locally recurrent or metastatic	Autologous DCs pulsed with E75 and E90 peptides with trastuzumab and vinorelbine	Ongoing
NCT00978913	I	14	Toxicity/ immune response	Metastatic	DCs transfected with survivin, hTERT and p53 mRNA with cyclophosphamide	Ongoing
NCT00622401	I/II	41	Toxicity	Metastatic	DCs/tumor cell fusion vaccine ± IL-12	Ongoing
NCT00715832	I	25	Toxicity	Metastatic	DCs loaded with oncofetal antigen/ iLRP	Ongoing
NCT01522820	I	30	Safety	Adjuvant	DCs/NY-ESO-1 fusion protein vaccine ± sirolimus	Ongoing
NCT00923143	I/II	57	Safety/immune response	DCIS	HER-2/Neu-pulsed DC vaccine	Ongoing
NCT00197522	I	5	MTD/toxicity	Metastatic	DCs infected with an adenovirus expressing Her-2	Completed
NCT00082641	I/II	24	Safety/toxicity/ immune response	Neoadjuvant or adjuvant	Adenovirus p53-infected DC vaccine ± chemotherapy ± RT	Ongoing
NCT00128622	I	24	Safety	Metastatic	Autologous DCs infected with CEA-6D-expressing Fowlpox-Trico	Completed
NCT00004604	I	24	Safety	Metastatic	CEA RNA-pulsed DC vaccine	Completed

Trials can be found at [48].
†Completed trials demonstrate actual number enrolled; ongoing trials demonstrate estimated enrollment.
DC: Dendritic cell; DCIS: Ductal carcinoma *in situ*; MTD: Maximum tolerated dose; pCR: Pathologic complete response; RT: Radiotherapy.

Table 1. Clinical trials for dendritic cell vaccine-based therapies (cont.).

NCT number	Study Phase	Estimated enrollment (n)†	Primary end point	Setting	Type of therapy (intervention)	Status
NCT00197925	I/II	40	Tolerability/ safety	Metastatic	Oncopeptide-loaded autologous DCs	Completed
NCT00107211	I	30	Feasibility/ safety/clinical response	DCIS neoadjuvant	HER-2/Neu-pulsed DC1 vaccine	Ongoing
NCT01431196	II	29	pCR	Stage II and III	Chemotherapy followed by DCs pulsed with tumor antigens	Ongoing
NCT00640861	II	45	Toxicity/ immune response	Stage II or III	MUC1/HER-2/Neu peptide DC vaccine	Ongoing
NCT00162929	I	5	Toxicity	Metastatic	DCs transduced by an adenovector expressing Her-2/ neu	Completed
NCT01782274	II/III	60	All-cause mortality	Metastatic	Allogeneic/ autologous hematopoietic stem cells, DCs and cytotoxic lymphocytes	Ongoing
NCT00003432	I/II	26	Immune response/ clinical efficacy	Metastatic	CEA RNA-pulsed DC vaccine	Ongoing
NCT00879489	I/II	24	Toxicity	Metastatic	Autologous DCs pulsed with human recombinant oncofetal antigen (OFP/iLRP)	Ongoing

Trials can be found at [48].
†Completed trials demonstrate actual number enrolled; ongoing trials demonstrate estimated enrollment.
DC: Dendritic cell; DCIS: Ductal carcinoma *in situ*; MTD: Maximum tolerated dose; pCR: Pathologic complete response; RT: Radiotherapy.

developed a novel *in vitro* DC-based vaccine against BC using adriamycin-induced apoptotic MCF-7 cells [4]. In this study the human BC cell line MCF-7, after 24-h treatment with adriamycin (5 μg/ml), was cocultured with healthy donor-derived iDCs. Treatment with adriamycin potentiates the immunogenicity of the MCF-7 BC cell line, leading to the induction of iDC maturation and T-lymphocyte activation *in vitro*.

Clinical studies in BC

These results have suggested a rationale to evaluate the role of DC-based vaccines in BC. The trials aim to demonstrate the safety and immunological/clinical response of this type of immunotherapy in various subtypes and settings of BC patients.

Brossart *et al.* analyzed the feasibility and efficacy of a vaccination approach using HLA-A2-restricted HER2 or MUC peptide-pulsed DCs in ten patients with metastatic BC and heavily pretreated advanced ovarian cancer [44]. No side effects were observed and immunologic responses were recorded in all patients, even in those heavily pretreated, suggesting that peptide-pulsed DC vaccinations could also be successfully used after intensive or even high-dose chemotherapy to eradicate residual disease. As DC vaccines are potentially limited by the relatively low number of identified tumor antigens and by their low immunogenicity, one strategy is based on the fusion of autologous tumor cells with DCs. Avigan *et al.* have proved that patients with metastatic breast and renal cancer vaccinated

with fusion cells generated from patient-derived tumor cells and autologous DCs showed immunological and clinical antitumor responses, with minimal toxicity [45]. Similar results were observed in patients with ER/PR-negative BC [46]. Approximately 58% of patients experienced a specific delayed type IV hypersensitivity reaction, as a result of immune activation, suggesting that tumor lyzate-pulsed DCs provide a wide source of BC antigens that are active in evoking anti-BC immune responses. The use of cytokine adjuvants, such as IL-12 or IL-2, might augment effectiveness of the DCs vaccine. A Phase I/II clinical trial evaluated the use of a DC vaccine and IL-2 in six metastatic renal and four BC patients [47]. Patients were treated twice with mature DCs pulsed with autologous tumor lyzate and low-dose IL-2. The vaccine was tolerable and vaccination induced specific immunity in all patients, despite response being observed in only one renal cancer patient, who achieved stable disease. Moreover, another Phase I/II trial is studying the safety of DC/tumor cell fusion when given together with IL-12 to see how well they work in treating women with stage IV BC (NCT0062240 [48]). Further studies are also assessing the potential of DC immunization to synergistically interact with other forms of medical treatment, such as chemotherapeutic compounds (e.g., vinorelbine or cyclophosphamide) or targeted therapy. The combination of two or more therapeutic strategies with different mechanisms of action may stimulate the immune system in different ways in order to evoke a strong and specific response to stop tumor cells from growing. Three clinical trials (NCT00088985, NCT00266110 and NCT00978913 [48]) are Phase II and I studies that are evaluating the efficacy and the toxicity of these combination therapies (Table 1).

Another promising approach to improve outcome of BC patients consists of targeting the innate and adaptive immune mechanisms. One strategy might be the use of autologous cytokine-induced killer cells (CIKs), which have shown significant cytotoxic activity in clinical studies [49]. Some investigators have assessed the combination of DCs with CIKs in 87 patients who underwent high-dose chemotherapy with docetaxel plus thiotepa. Compared with 79 patients who received standard-dose chemotherapy, in the high-dose chemotherapy group progression-free survival and overall survival were improved, demonstrating that the combination of high-dose chemotherapy with DCs/CIKs can be an effective choice for selected metastatic BC patients [50].

Limits of DC vaccines

Immunotherapy with DCs represents a very attractive therapeutic approach in the management of BC. Despite these cells seeming to be effective in inducing a detectable tumor antigen-specific immunity and DCs vaccines being well tolerated and safe, clinical benefit is still disappointing. Several reasons may explain the unsatisfactory result of this therapeutic strategy and the difficulty in developing DC-based therapies effective in controlling BC.

Time to vaccination & evaluation of response

Data from the preclinical models suggest that vaccinations are more effective in the prevention of tumor growth rather than in the treatment of established tumors [51]. For this reason, although the majority of DC vaccine clinical trials were performed in patients with large tumor burden and/or advanced disease with disappointing clinical results, we believe that clinical benefit could be reported in patients with disease remission or with small tumor burden. Studies have been conducted in patients with early BC and carcinoma *in situ*, in adjuvant and neoadjuvant settings [52–54]. In one of them, 27 patients with HER2 overexpressing ductal BC *in situ* were enrolled in a neoadjuvant immunization trial [54]. A DC vaccine was administered before surgical resection of carcinoma. After surgery, in 11 out of 22 (50%) subjects with residual ductal carcinoma *in situ*, vaccination induced decline and/or eradication of HER2 expression, showing that even in the presence of an early tumor DCs are potent inducers of immunity against HER2 cells. We could take advantage of the results of ongoing studies using HER-2-pulsed DCs (NCT00923143, NCT00107211), adenovirus p53-infected DCs (NCT00082641), DC/NY-ESO-1 fusion protein vaccine (NCT01522820) and MUC1/HER-2/Neu peptide (NCT00640861 [48]) to assess the real utility of this strategy in this setting. However, randomized studies are required to establish whether immunotherapy provides an additional benefit to standard therapy.

How to assess clinical response is a major and debated issue in all immunotherapies. Indeed, to determine the clinical efficacy in immunotherapy-based trials traditional clinical/radiological criteria (Response Evaluation Criteria In Solid Tumors [RECIST]) based on tumor size are still used, even if these criteria seem to be inappropriate, since eliciting antitumor immune responses is slow and often associated with an increase in tumor mass, due to immune cell infiltration rather than with a reduction in tumor burden. Thus, novel criteria are required to capture the antitumor responses with immunotherapeutic agents, as proposed by some researchers [55]. At the moment, overall survival might be the only objective parameter to calculate the clinical efficacy of immunotherapy, but given that the evaluation of overall survival may require a long time, surrogate markers of overall survival are needed [56].

Technical & immunological issues

The technical problems during the procedure of DC generation together with imperfect antigen presentation, due to use of defective DCs and/or ineffective TAAs, may have contributed to the failure of this therapy.

The most common approach used to collect DCs is the collection of peripheral blood mononuclear cells obtained from whole blood or leukapheresis [57]. This method does not allow the selective harvesting of monocytes, but other cells may contaminate the collection. Also, after leukapheresis, cells must be subjected to other procedures for their isolation, selection and differentiation in DCs. All these processes, although necessary, can have an important impact on the number and quality of the obtained DCs. Moreover, it has yet to be understood which signals and combination of stimuli for *ex vivo* manipulation make DCs mature/immunogenic [26]. The maturation of DCs is a critical process and the risk is that DCs, not adequately stimulated, could be in an immature state, thus inhibiting rather than inducing an immune response [18]. Therefore, if on the one hand the *ex vivo* manipulation of DCs is a valid way to employ these cells in cancer immunotherapy, on the other hand, many problems must yet be overcome to obtain proper cells to develop an adequate immune response. This may require the transfer of genes encoding costimulatory molecules or cytokines into DCs to enhance the binding of tumor antigens to MHC molecules or to TLRs. Furthermore, since many tumor-derived factors can limit DCs differentiation and maturation (e.g., PD-L1 or VEGFR-1) [58], the association of DCs and other molecules capable of increasing antitumor efficacy could improve therapeutic effects of vaccination. For example, it could be interesting to use DC-based vaccination in combination with a PD-L1 inhibitor or with anti-VEGFR antibodies, or with other drugs directed to immunosuppressive molecules (i.e., TGF-β, IL-10 and IL-6) or signaling pathways such as STAT3, MAPK, β-catenin that interfere negatively with the immune response by preventing tumor growth and stimulating an effective and adequate immune response to eradicate malignant cells [59].

An alternative could be to target antigens directly to the DCs *in vivo*. This strategy stimulates the activation of natural DCs *in vivo* via monoclonal antibodies specific for particular DC surface molecules [60,61]. This system represents a promising approach [62,63]. However, despite various efforts, further studies are needed to establish which methods allow to obtain the best functional DCs able to improve immune response.

As discussed before, several studies conducted in BC have confirmed the impaired function of these immune cells. An enzyme that could be involved in the induction of immune tolerance instead of immune response is IDO, the accumulation of which in DCs has been observed in lymph nodes of patients with melanoma and BC and may precede the development of lymph node metastases [64]. A recent study reporting on the immunization of breast tumor-bearing mice with DCs loaded with tumor antigens and with siRNA-silenced IDO expression showed enhanced tumor antigen-specific T-cell proliferation and CTL activity, suggesting that silencing of IDO is an effective strategy to improve the efficiency of DC-based cancer immunotherapy [65].

Moreover, another problem is that only certain identified antigens can induce an immune response followed by cancer elimination. A number of breast tumor antigens have been described, and HER-2, carbohydrate antigens, MUC-1, CEA, p53 and cancer-testis antigens (NY-eso-1), have received the greatest attention as antigens for vaccine formulation [66]. New information revealed by the genomic and proteomic classification of BC should help us to clarify the specific biologic types of BC with different levels and patterns of tumor antigen expression and to identify new specific tumor antigens for effective immunotherapy. The use of DCs provides an opportunity to overcome the relative nonimmunogenicity of BC and address the underlying immunodeficiency. The ideal specific antigen should be overexpressed on the tumor cells and should have limited distribution in normal tissue, even if DC vaccines targeting single antigens have not often led to a measurable immune response because of the tumor escape mechanisms [14]. As discussed before, DCs may be directly loaded with autologous BC cell lyzates or apoptotic bodies, thus allowing the presentation of multiple tumor antigens [45,46]. However, vaccination with the whole cells expressing tumor-specific antigens but also non-tumor-specific antigens could induce tolerance to the antigens contained in the vaccine, instead of eliciting immune response against TAAs. Transfection of DCs with amplified tumor-derived RNA or DNA might represent a potential solution [67] and improvement of this technique might translate into better outcome. Thus, the success of future DC vaccines in BC will depend on the identification of additional immunogenic antigens, on developing the best antigen delivery systems and on the elucidation of the entire network of immune signaling pathways that regulate immune responses in the tumor microenvironment. Only by doing that it will be possible to develop a personalized immunological therapy, based on the specific characteristics of individual patient's immune system and on the antigenic tumor profile.

Conclusion

DC-based immunotherapy is a promising therapeutic approach for BC patients. Currently, several DC-based vaccine strategies are being developed both at the preclinical stage and in clinical trials. DC vaccines have shown to be able to induce antigen-specific immunity *in vivo*, but so far active specific immunotherapy with DCs for BC does not seem to produce a significant clinical benefit. Several questions are still open, such as how DCs and lymphocytes work, which alterations of immune response occur in the tumor microenvironment, which is the best setting for the use of DCs and how we can best use them in immunotherapy. Molecular typing of BC and genomic identification of BC antigens, as well as combinatorial therapies that target both BC-specific immune activation and inhibition of immune tolerance, could be also useful to improve the specific response of vaccines. Thus, a better understanding of the complex interplay among the host immune response, tumor cells, tumor microenvironment and further studies on tumor immunology are warranted to determine whether DC vaccination, alone or in combination with other therapies, could become a successful approach to improve clinical outcome and to control BC.

Future perspective

Although at the moment the success of this approach has been limited, in the future we believe that both the improving of the procedures for the *in vivo* or *ex vivo* manipulation of DCs and the promising information derived from the molecular typing of BC and genomic identification of BC antigens could lead to extend the benefit of DC-based immunotherapy to a larger BC patient population. The combinatorial approaches could be useful to capitalize on the effectiveness of vaccines. Drugs directed against immunomodulatory mechanisms that restrict the antitumor response could enhance the efficacy of DCs vaccines in BC.

Some molecules such as PD-1/PDL-1, CTLA-4 and immune cells such as Tregs are involved in the induction of tolerance to antigens and their upregulation is associated with increased risk of developing BC [68]. Investigators have evaluated the efficacy of anti-CTLA-4 or anti-PD1/PDL-1 in BC, but so far none of these drugs alone have proven to be an effective approach [69].

The administration of anti-CTLA-4 blocking monoclonal antibodies in previously DC-vaccinated advanced melanoma and ovarian cancer patients increased immune-mediated tumor destruction in some subjects [70]. Similar results could be obtained in BC patients. The major problem is management of

Executive summary

Background

- Despite breast cancer (BC) traditionally being considered as poorly immunogenic, immunotherapy is an emerging and promising new approach to treat this cancer.

Immune system & cancer

- Immune response is important to eliminate cancer cells, but sometimes cancer cells can avoid the immune system allowing tumor growth.
- Active immunotherapy in BC enables the immune system to discover neoplastic growth and to reject transformed cells. The use of dendritic cells (DCs) is now considered one of the most promising approaches in cancer immunotherapy, due to their notable ability to stimulate T-cell response.

DC biology & DC-based vaccines

- DCs are the most effective immunogenic APC.
- In cancer patients DCs present abnormalities that make antitumor T-cell activation difficult.
- In order to improve the immune function of these cells, it would be better to utilize DCs manipulated *ex vivo*.

Preclinical & clinical studies with DC-based vaccines

- In preclinical models, DCs have been shown both to inhibit the growth of BC and to provide a memory response to tumor antigens.
- Many clinical trials were performed in BC in order to demonstrate the safety and immunological/clinic response of this type of immunotherapy in various subtypes and in diverse settings of patients.

Limits of DC vaccines

- Patients enrolled in the majority of clinical trials present advanced disease.
- Novel criteria are required to define the antitumor responses with immunotherapeutic agents.
- The technical problems during the procedure of DC generation together with the use of defective DCs and/or ineffective tumor-associated antigens may have contributed to the failure of this therapy.

Conclusion & future perspective

- Molecular typing of BC and genomic identification of BC antigens, as well as combinatorial therapies that target both BC-specific immune activation and inhibition of immune tolerance, could be also useful to improve the specific response of DC vaccines.

the resultant side effects, which are often serious, and the autoimmune manifestations that occur in many patients. Thus, effort is needed to clarify the immunological and clinical effects of this form of immunotherapy in vaccinated patients to reduce toxic effects as well as improve the antitumoral response.

Another option to enhance the efficacy of DC-based therapy in BC could be to combine it with other therapies such as chemotherapy or radiotherapy. The tumor apoptosis and/or necrosis induced by radiotherapy and some types of chemotherapeutic drug (e.g., anthracyclins, cyclophosphamide and platinum compounds) releases large amounts of tumor-associated proteins, can promote DC activation by molecularly defined pathways and depletes Tregs, potentially enhancing immune responses [71]. For example, anthracyclines and oxaliplatin promote tumor antigen presentation by DCs though the translocation of CRT on the tumor cell surface, postapoptotic release of the chromatin-binding protein HMGB1 and extracellular release of ATP [72]. Recent evidence also suggests that targeted therapies with small inhibitors may also benefit from antitumor immune responses. One ongoing Phase III trial will assess a DC vaccine in subjects with advanced kidney cancer as an add-on to targeted therapy with sunitinib, a receptor TKI (NCT01582672; ADAPT trial [48]). The combined use of this or other small-molecule inhibitors and immunotherapy might be synergistic and might improve the antitumor effects [73]. We expect to get encouraging results from ongoing studies in order to determine whether immunotherapy in combination with other therapeutic strategies can provide an additional benefit with no significant side effects and also to identify the immunological features of patients that best respond to DC-based anticancer vaccines (Table 1) [48].

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