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Zoledronate-pulsed dendritic cell-based anticancer vaccines

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The addition of zoledronate to tumor-associated antigen (TAA)-loaded dendritic cells (DCs) promotes the activation of interferon γ-secreting Vγ9 γδ T cells, in turn eliciting TAA-specific CD8+ T-cell responses. Immunological responses induced by zoledronate-pulsed DC-based vaccines have been associated with therapeutic effects in clinical trials.

Dendritic cell (DC)-based vaccines relying on several distinct methods for antigen loading have been developed for cancer immunotherapy.[1] In this context, it has been shown that Vγ9 γδ T cells activated by zoledronate can link the innate and adaptive arms of the immune system via DCs so to boost the activation of tumor-associated antigen (TAA)-specific CD8+ cytotoxic T lymphocytes (CTLs).[2] TAA-pulsed immature DCs (imDCs) combined with zoledronate activate Vγ9 γδ T cells while promoting the expression of CD40 ligand (CD40L) on their surface and the secretion of cytokines such as interferon γ (IFNγ), ultimately driving the expansion of TAA-specific CD8+ CTLs. We postulate that CD40L on imDC-activated Vγ9 γδ T cells in combination with zoledronate and Vγ9 γδ T cell-derived cytokines stimulate the functional maturation of imDCs, which is accompanied by the upregulation of chemokine (C-C motif) receptor 7 (CCR7) and CD62 ligand (CD62L) and promotes the proliferation of TAA-specific CD8+ CTLs.

We employed zoledronate-pulsed DCs to develop vaccines targeting various TAAs and characterized them in clinical settings (**Fig. 1**). In particular, we performed a Phase I/IIa clinical trial involving elderly acute myeloid leukemia (AML) patients and DCs pulsed with an HLA-A2402-restricted Wilms' tumor 1 (WT1)-derived peptide and zoledronate.[3] Three elderly HLA-A2402+ AML patients were enrolled in this study, 2 of which developed a WT1-specific immune response, as documented by skin delayed-type hypersensitivity (DTH) and/or IFNγ ELISPOT assays. Along with the elicitation of WT1-targeting immune responses, either a transient decrease in leukemic cells or disease stabilization was observed in these 2 patients. Unfortunately, the other patient dropped out of the study after the third round of vaccination owing to the rapid outgrowth of leukemic cells, correlating with the absence of WT1-specific immune responses. Recently, we employed DCs pulsed with zoledronate and an overlapping pool of WT1-derived peptides for the treatment of patients with WT1-expressing solid tumors (UMIN-CTR ID 000009447), because both CD8+ CTLs and CD4+ helper T cells targeting WT1 might (at least potentially) be induced even in the absence of HLA restriction.

It is well known that tumor-specific CD8+ CTLs are effectively activated once they recognize TAAs presented in the context of MHC class I molecules along with appropriate co-stimulatory signals. Experiments in which DCs were loaded with purified TAAs or TAA-coding mRNA revealed that antigens can be presented on both MHC class I and class II molecules, depending on the method of antigen delivering (which influences its intracellular processing).[4,5] We have previously studied electroporation (EP) as a way to deliver TAAs to DCs while preserving their viability. In fact, antigen-specific CD8+ CTLs are elicited more effectively by DCs that are electroloaded with antigens than by immature DCs that are simply cultured in the presence of antigens.[6] By the closed-flow EP system, we succeeded in loading zoledronate-pulsed DCs with autologous tumor cell lysates while maintaining the viability. In a Phase I clinical trial, mild adverse events were documented when this DC vaccine was used for the treatment of advanced/recurrent cancer patients, or as an adjuvant therapy for some other types of neoplasms. However, no autoimmune responses developed upon the administration of the autologous tumor cell lysate-loaded DC-based vaccine. Among 41 patients affected by different solid tumors, vaccination was associated with an overall response rate of 4.9% and a clinical benefit rate of 31.7%. Of note, more than 90% of the patients exhibiting a clinical benefit upon vaccination manifested DTH responses. Thus, the antitumor effects of our autologous tumor cell lysate-loaded

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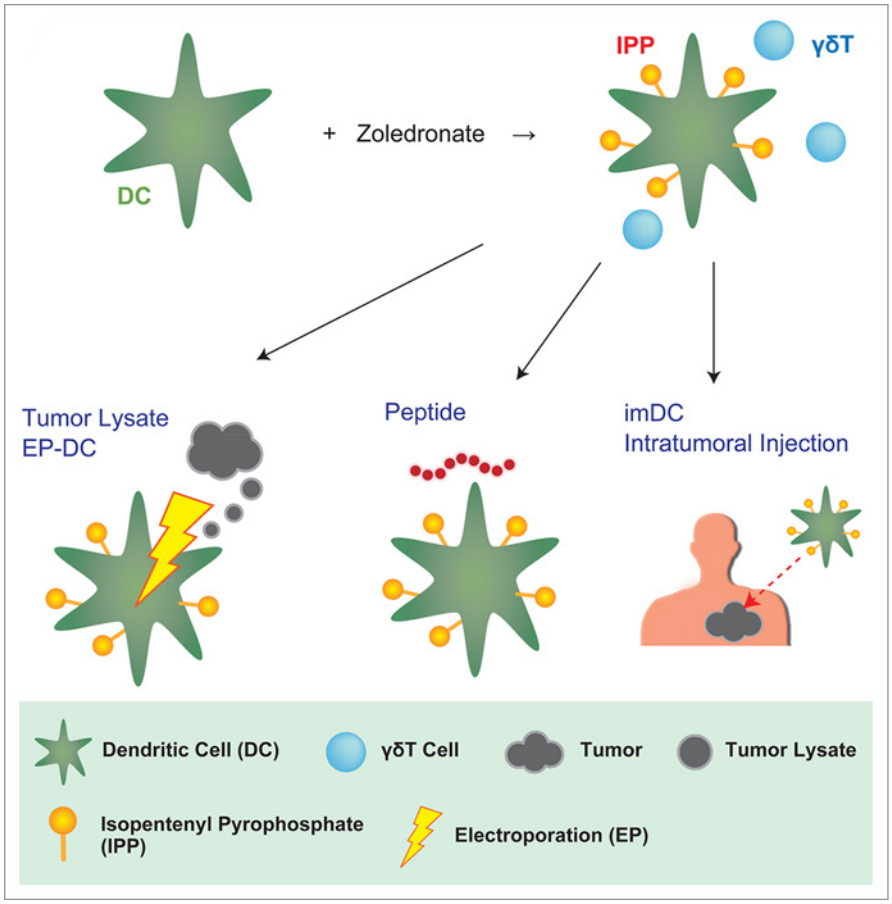

Figure 1. Zoledronate-pulsed dendritic cell-based cancer vaccines. Zoledronate is an aminobisphosphonate that induces the accumulation of isopentenyl pyrophosphate (IPP), a ligand of the Vγ9 γδ T-cell receptor (TCR), in a variety of cells. Vγ9 γδ T cells activated by zoledronate can link the innate and adaptive branch of the immune system via dendritic cells (DCs), hence supporting the activation and proliferation of tumor-associated antigen (TAA)-specific $CD8^+$ cytotoxic T lymphocytes (CTLs). DCs can be effectively loaded with purified TAAs, cancer cell lysates or mRNA preparations by a closed-flow electroporation (EP) system. Alternatively, loading can be performed by culturing DCs in the presence of 9–15-mer TAA-derived peptides. When it is difficult to obtain adequate amounts of TAAs for loading DC ex vivo, tumor-specific immune responses might be induced in vivo, upon the injection of immature DCs (imDCs) into neoplastic lesions.

DC-based vaccine appear to be intimately associated with elicitation of tumor-specific immune responses.[7]

In some circumstances, it is difficult to obtain adequate amounts of TAAs for loading DCs ex vivo. In this settings, strategies that efficiently elicit tumor-specific immune responses in vivo, upon the injection of autologous DCs in neoplastic lesions that have previously been exposed to radiotherapy or chemotherapy, would be highly desirable.[8] DCs can indeed efficiently engulf and process extracellular antigens shedding from apoptotic cells.[9] OK432, a lyophilized preparation of a poorly virulent strain of *Streptococcus pyogenes* killed by penicillin, is widely used as a maturation stimulus for DCs. In a previous study, OK432-pulsed imDCs were administered intratumorally—by the endoscopic ultrasound (EUS) technique—to patients with locally advanced pancreatic cancer, aimed at potentiating the antineoplastic activity of systemic gemcitabine and conventional lymphokine-activated killer cells stimulated with anti-CD3 monoclonal antibodies.[10] Three out of 5 patients enrolled in this clinical trial manifested objective clinical responses: (1) a partial remission and (2) disease stabilization lasting for more than 6 mo. In the patient undergoing partial remission, OK432-pulsed imDCs combined with gemcitabine had elicited TAA-specific CTLs, as demonstrated by IFNγ ELISPOT assays. Currently, we inject zoledronate-pulsed imDCs, instead of OK432-pulsed imDCs, into neoplastic lesions, following the protocol that was previously defined for locally advanced pancreatic cancer patients.

Of note, no serious treatment-related adverse events were documented in clinical trials involving zoledronate-pulsed DCs loaded with various TAAs. The success of cancer immunotherapy depends on the targeting of adequate TAAs and on the choice of an immunization strategy that elicits robust tumor-specific immune responses. Zoledronate-pulsed DC-based cancer vaccines represent a promising immunotherapeutic approach for the treatment of various neoplasms. This strategy is supported by recent technological progresses, such as the possibility to generate pools of overlapping TAA-derived peptides or the closed-flow EP system for the loading of DCs with tumor cell lysates.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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