Dendritic cell-derived exosomes for cancer immunotherapy: hope and challenges

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Dendritic cells (DCs) are the most potent antigen-presenting cells in the human body. When primed with antigens, DCs activate both helper and killer T cells and B cells. In the past decade, DC-based vaccine has emerged as an important strategy for cancer immunotherapy (1). Several phase I or II clinical trials of DC-based immunotherapy to treat different cancers have been reported with increased survival and mild vaccination-related side effects (2,3). However, DC-based immunotherapy has several limitations. For example, molecular composition of DCs may change and is difficult to be defined (4). Tumor cells may secret soluble immunosuppressive cytokines that could convert immature DCs into tolerogenic DCs, which may activate Treg cells (5). Live DCs are inconvenient for storage (4) and have to be produced locally with quality control issues.

To address these challenges, DC-derived exosomes (dexosomes or Dex) have been developed as an alternative approach of cancer vaccines (4). Exosomes are cell-derived vesicles of endosomal and plasma membrane origin, which are released into extracellular environment. These extracellular vesicles were first discovered in the immature red blood cells in 1987 (6) and represent an important means for intercellular communication. B cells were reported to secrete antigen-presenting exosomes capable of inducing antigen-specific MHC class II-restricted T cell response in 1996 (7). Subsequently, Zitvogel *et al*. described that DCs also secrete antigen-presenting exosomes (8). Both immature and mature DCs can release

exosomes. Dex possess many molecules necessary for antigen presentation, such as MHC class I, MHC class II, and costimulatory and adhesion molecules (9). Tumor peptide-pulsed Dex activate antigen-specific cytotoxic T lymphocytes (CTLs) *in vivo* to eradicate or suppress growth of established murine tumors in an MHC- and CD8$^+$ T cell-dependent manner (8). Thus, exosome-based cell-free vaccines represent an important strategy to suppress tumor growth.

In a recent phase II clinical trial published in *Oncoimmunology* (10), Besse *et al*. attempted to use Dex as maintenance immunotherapy after chemotherapy cessation for patients with non-small cell lung cancer (NSCLC). They reported that Dex boosted antitumor activity of natural killer (NK) cells. In this study, they prepared second-generation Dex by differentiating patient's monocytes into immature DCs with GM-CSF and IL-4, followed by induction of mature DCs with INF-γ and loading of tumor-associated antigenic peptides. IFN-γ was reported to induce the expression of costimulatory molecules and ICAMs in DCs (11). Compared to the first-generation INF-γ-free Dex for phase I trial (12), INF-γ-Dex prepared from peptide-loaded mature DCs exhibited a mature phenotype with upregulated expression MHC class II molecules, tetraspanins, CD40, CD86 and ICAM-1/CD54. These data suggest that INF-γ treatment has the potential to improve the capacity of Dex for antigen presentation and T cell activation, as previously reported (11).

The phase II trial was to investigate whether INF-γ-Dex as maintenance immunotherapy can improve the rate of progression-free survival (PFS) at 4 months after chemotherapy (10). This study enrolled 22 patients with advanced NSCLC, all of which had received four cycles of platinum-based chemotherapy. Before the Dex trial, 14 patients showed stabilization, while eight patients experienced a partial response. Patients were administered metronomic oral cyclophosphamide to reduce Treg cell function and stimulate dual INF-γ/IL-7-producing T cells before intradermal injection of INF-γ-Dex. Presumably, this may facilitate Dex-mediated T cell priming and restore T cell and NK cell functions.

The results showed that seven patients (32%) remained stable after nine injections. The PFS rate at 4 months was 32%, and the median PFS for all 22 patients was 2.2 months. There was no objective tumor response according to the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. The median overall survival (OS) for all patients was 15 months with a survival rate at 6 months of 86%. One patient developed a grade three hepatotoxicity, and there was no treatment-related death. Thus, Dex are well tolerated.

Despite the intention behind the development of the second-generation Dex, the study failed to detect T cell response (10). However, an increase in NK cell functions was observed in a fraction of these NSCLC patients. Importantly, MHC class II expression levels of the final INF-γ-Dex product correlated with expression levels of the NKp30 ligand BAG6 on Dex, and with NK functions. NK cell activation in turn was associated with longer PFS. Interestingly, a potential increase in NK cell lysis ability was also observed in a phase I clinical trial of INF-γ-free Dex (13).

This phase II trial showed only limited efficacy for Dex immunotherapy. The primary endpoint of the trial was to observe at least 50% of patients with PFS at 4 months after chemotherapy cessation (10). However, this objective was not achieved. Several reasons may explain why Dex therapy had limited efficacy. First, cancer antigens loaded onto exosomes might not be clinically relevant due to the heterogeneity of the patient cohorts. Second, INF-γ used in the process of Dex production may upregulate programmed death ligand-1 (PD-L1) on DCs and Dex (14). Finally, the limited efficacy may be attributed to the lack of adaptive immune response, particularly antitumor CD8+ T cell response.

Several strategies may improve the efficacy of Dex immunotherapy for future clinical trials. A previous study indicated that protein-loaded Dex, but not peptide-loaded Dex, induced CTL response and inhibited tumor growth, both of which require the activation of CD4+ and B cells (15). Therefore, full-length tumor-associated antigens (TAAs) may be used to replace their peptides to promote the activation of helper T and B cells, which may in turn boost CD8+ T cell response. Dex loaded with multiple TAAs may expand the coverage for the heterogeneity of human tumors. Mutated TAAs could be used to improve T and B cell response (16). Dex may be engineered to upregulate the expression of costimulatory molecules. Engineered Dex could also carry mRNAs coding for relevant TAAs or activation molecules. The expression of PD-L1 and PD-L2 on Dex should be quantified and downregulated. Immunotherapies to block immune checkpoint molecules, such as CTLA-4 or PD-1, can enhance lymphocyte response (17) and could be used in combination with Dex to boost antitumor T and B cell response. A previous phase I clinical trial showed that ascites-derived exosomes (Aex) in combination with GM-CSF, but not Aex alone, induced beneficial tumor-specific CTL response (18). Combination therapy of Dex with GM-CSF may further elicit antitumor CTL response. These strategies could improve the outcomes of future clinical trials with Dex.

Despite the drawbacks and challenges, the study by Besse *et al.* is the first phase II clinical trial of Dex. As a new vaccine strategy for cancer immunotherapy, Dex remain promising with potential for improvement. The prospect of Dex therapy is encouraged by the activation of NK cells (10) as well as the promising outcomes of clinical trials with DC-based vaccines (1). Future basic research and clinical trials should focus on how to stimulate CTL response by Dex. The successful development of Dex-based immunotherapy will further expand the weaponry to battle cancer.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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