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ORIGINAL ARTICLE

Exploitation of Langerhans cells for *in vivo* DNA vaccine delivery into the lymph nodes

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There is no clinically available cancer immunotherapy that exploits Langerhans cells (LCs), the epidermal precursors of dendritic cells (DCs) that are the natural agent of antigen delivery. We developed a DNA formulation with a polymer and obtained synthetic 'pathogen-like' nanoparticles that preferentially targeted LCs in epidermal cultures. These nanoparticles applied topically under a patch-elicited robust immune responses in human subjects. To demonstrate the mechanism of action of this novel vaccination strategy in live animals, we assembled a high-resolution two-photon laser scanning-microscope. Nanoparticles applied on the native skin poorly penetrated and poorly induced LC motility. The combination of nanoparticle administration and skin treatment was essential both for efficient loading the vaccine into the epidermis and for potent activation of the LCs to migrate into the lymph nodes. LCs in the epidermis picked up nanoparticles and accumulated them in the nuclear region demonstrating an effective nuclear DNA delivery *in vivo*. Tissue distribution studies revealed that the majority of the DNA was targeted to the lymph nodes. Preclinical toxicity of the LC-targeting DNA vaccine was limited to mild and transient local erythema caused by the skin treatment. This novel, clinically proven LC-targeting DNA vaccine platform technology broadens the options on DC-targeting vaccines to generate therapeutic immunity against cancer.

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INTRODUCTION

Innovative vaccines to treat cancer are designed to target dendritic cells (DCs), the main inducers and regulators of the immune system.[1] Epidermal Langerhans cells (LCs) are DCs specialized in the delivery of antigens to lymph nodes in order to induce cytotoxic T-lymphocytes (CTLs) capable of specific killing of tumor cells.[2–6] Relatively few vaccines are administered to the epidermis and there is no vaccine that effectively and selectively targets the LCs before they migrate to the lymph nodes.[7] Vaccines that express antigens from a plasmid DNA (pDNA) do not require cross-priming to induce CTL responses. Therefore, preferential delivery of pDNA to LCs may result in more robust CTL responses than priming these cells with peptides or proteins.[7,8] Unfortunately, inefficient uptake and intracellular degradation of pDNA limits the efficacy of both antigen expression and induction of CTL responses.[9]

We present here the mechanism of action of a clinically proven vaccine technology platform that was developed to harness the immunological function of LCs, to transport antigens from the periphery into the lymph nodes and induce robust CTL responses. The underlying premise of this approach is provided by *ex vivo* manipulation of the DCs that generates optimally activated antigen-presenting cells and a superior method for stimulating clinically significant antitumor and antiviral immunity as compared with more traditional vaccination methods. The platform technology has two key components: (1) LC-targeting nanoparticles that optimized for cellular entry and nuclear delivery of the pDNA for potent expression of antigens; (2) the nanoparticle vaccine formulation is combined with a medical device developed for *in vivo* LC-targeting vaccination. The first LC-targeting vaccine (DermaVir) was safe and induced potent and long-lasting memory CTL responses in human subjects as tested in three clinical trials for infectious disease indications.[10–12] Any DNA incorporated into nanoparticles would induce similar antigen-specific CTL responses because the physico-chemical properties of the LC-targeting nanoparticles are independent on the sequence and size of the encapsulated pDNA.[13] Our translational studies bring corroborating evidences that this technology safely and efficiently deliver pDNA-encoded antigens to the nucleus of LCs, increases their activation and their motility, facilitating antigen delivery to the lymph nodes. Finally, we demonstrate practical and inexpensive clinical applications of the LC-targeting vaccine as an *in vivo* alternative to DC-targeted immunotherapy developed against infectious, neoplastic and autoimmune diseases.

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