

# Targeting dendritic cells in situ for breast cancer immunotherapy

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We have recently developed a protein vaccine against breast cancer in which HER2 is delivered to dendritic cells (DCs) in vivo through receptors expressed on their surface. Our results indicate that this is a promising approach to induce durable, broad and integrated immunity against breast cancer.

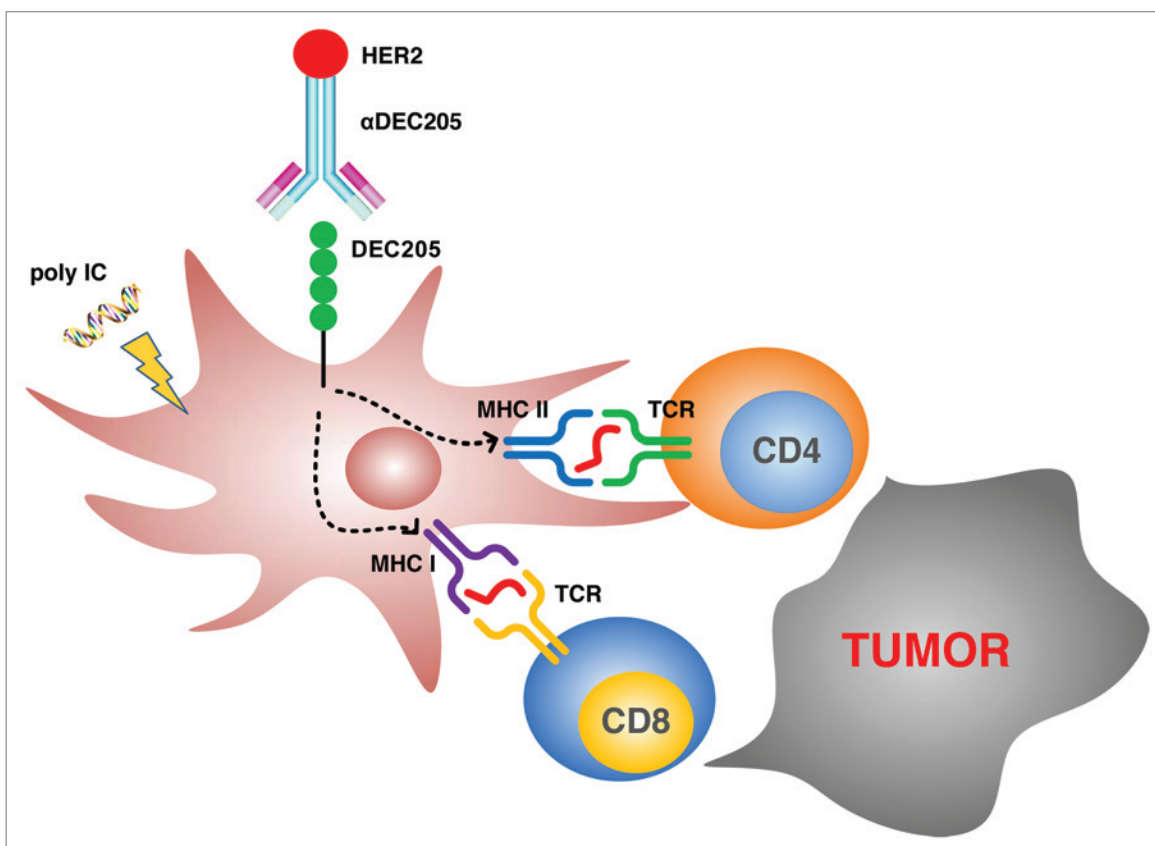
Great advances have been made to improve remission rates in breast cancer patients. However, it is necessary to search for new treatment strategies to reduce the risk of relapse, which today remains high. HER2 is a protein that is overexpressed in a sizeable fraction of breast cancers, hence constituting an attractive target for immunotherapy. For instance, Herceptin (trastuzumab) is a licensed monoclonal antibody that binds to the extracellular domain of HER2.<sup>1</sup> Although HER2 is expressed by malignant cells as a non-mutated self-antigen, immune responses can be elicited by vaccination. Compared with passive immunotherapy with trastuzumab, active vaccination has several advantages. Thus, unlike antibody therapy, vaccination induces more durable protection, particularly through vaccine-induced T cells that require relatively low levels of HER2 expression for recognizing cancer cells. Only small amounts of peptide-MHC complexes are required for T-cell recognition, and there is evidence that a large proportion of breast cancers, while not sensitive to trastuzumab, still express HER2 levels that are sufficient for T-cell recognition. Therefore, active vaccination has the potential to address a larger population of women than anti-HER2 antibodies, which target patients with high HER2 expression levels. In addition, because vaccine-induced immunity is durable, strong and distinct in its mechanism, vaccines are

a logical way to improve resistance to the development of (micro)metastasis.

Protein vaccines are an attractive platform for cancer vaccines because they can easily be manufactured and administered repeatedly. However, the soluble HER2 protein as a vaccine only shows weak immunogenicity, and has usually failed to confer protection against HER2-expression tumors. Our focus was hence to directly deliver HER2 protein to dendritic cells (DC) in vivo. DCs are potent antigen-presenting cells and are capable of processing the HER2 protein to liberate peptides for presentation on MHC Class I and II complexes to CD8<sup>+</sup> and CD4<sup>+</sup> T cells, respectively (Fig. 1). In addition, DCs are immune-initiating cells that are able to find in vivo rare clones of HER2-specific T cells, expanding them and inducing critical helper and killer anticancer T cell functions. Discovery of antigen uptake receptors on DCs has enabled the engineering of monoclonal anti-receptor antibodies that are efficiently and specifically delivered to DCs in situ, with no need for DC isolation as in previous DC-based vaccines.<sup>2</sup> One of the receptors that were targeted in our recent study is DEC205, a Type I C-type lectin that is abundant on CD8 $\alpha$ <sup>+</sup> DCs.<sup>3</sup> Ralph Steinman and his collaborators have developed an efficient DC-delivery system via DEC205, which after extensive preclinical studies in mice is now being tested in proof

of concept studies in volunteers.<sup>4</sup> Studies in mouse models have demonstrated that a foreign antigen, such as the HIV gag p24 protein, when targeted to DCs by an anti-DEC205 antibody, is efficiently processed and presented to T cells. In fact, antigen delivery through DEC205 increases the efficiency of antigen presentation relative to non-targeted antigens over 100-fold, and it induces both CD4<sup>+</sup>/CD8<sup>+</sup> T-cell and antibody responses. An ongoing study in healthy volunteers has demonstrated that an anti-DEC205-p24 vaccine administered together with polyriboinosinic:polycytidylic acid (polyI:C), a synthetic double-stranded RNA as adjuvant, elicits an integrated B- and T-cell immunity.

Recently, we provided proof of concept evidence—in a preclinical setting—in a way to load DCs with the HER2 protein and to stimulate the immune system to enhance the antitumor immunity initiated by DCs.<sup>5</sup> One of the major challenges in DC-based cancer vaccination is to include in the vaccine protocol an adjuvant to optimize DC maturation. Emphasis is now being placed on microbial mimics as adjuvants, for example agonists of innate microbial recognition receptors such as Toll-like receptors (TLRs).<sup>6</sup> We found that the TLR3 ligand polyI:C is a superior adjuvant for inducing T-cell immunity in mice, as within 4 h of injection DCs mature to become immunogenic.<sup>7</sup> We showed that TLR agonists are



**Figure 1.** Induction of T-cell immunity against breast cancer through a HER2 protein vaccine targeted to dendritic cells (DCs) in vivo. Maturation of DCs is induced by the Toll-like receptor 3 (TLR3 = ligand polyI:C. HER2 protein is delivered to DCs via an anti-DEC205 hybrid antibody). DCs are able to process the HER2 protein to liberate peptides for presentation on MHC Class I and II complexes to CD8<sup>+</sup> and CD4<sup>+</sup> T cells, respectively.

required to elicit tumor antigen-specific T-cell responses when mice were injected with DEC205-HER2 hybrid monoclonal antibody. We demonstrated that the HER2 protein—when delivered through DEC205—induces broad, potent, multifunctional and durable Th1 responses, as well as CD8<sup>+</sup> T-cell and B-cell responses. Such integrated immunity is desirable for tumor immunotherapy. Furthermore, using a mouse transplantable tumor model, we demonstrated that the DEC205-HER2 vaccination provides significant long-term survival benefits to mice challenged with *neu*-expressing tumors. This protection is mediated by both CD4<sup>+</sup> and CD8<sup>+</sup> T cells, with CD8<sup>+</sup> T cells playing a more dominant role.

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