

# Targeting immune evasion in cancer immunotherapy

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## Abstract

Important advances in the fields of immunology and tumor biology are fueling the development of new immunological approaches to treat cancer. The premise of active immunotherapy, cancer vaccination, is that immunizing against tumor antigens will control tumor growth. Murine studies have shown that immunizing with dendritic cells (DC) loaded with tumor antigens is an effective method of stimulating CD4+ and CD8+ T-cell responses and inducing antitumor immunity. A simple and broadly applicable method to load DC with tumor antigens is transfection with the corresponding mRNA. Comparative studies have shown that mRNA transfected DC are highly effective in stimulating T-cell immunity *in vitro* and *in vivo*, in general more effective than DC loaded with conventional forms of antigens such as peptides, proteins, vaccinia vectors or plasmid DNA. Clinical trials in patients with prostate and renal cancer have established the safety and feasibility of treating cancer patients with mRNA transfected autologous DC. The striking observation and the hallmark of the clinical experience to date is that the majority of the patients vaccinated with diverse antigens such as PSA, TERT or total RNA, responded immunologically to the vaccinating antigen. Strategies to improve the magnitude and persistence of the vaccine-induced immune responses are explored in two-arm clinical trials. For example, a clinical trial in patients with prostate cancer has evaluated a method to enhance the induction of TERT-specific CD4+ T-cell responses in which one cohort of patients is vaccinated with a modified TERT (LAMP-TERT) mRNA-encoded antigen that is redirected into the class II presentation pathway. Another clinical trial, in patients with renal cancer vaccinated with autologous tumor RNA transfected DC, evaluated the benefits of removing regulatory T cells prior to vaccination and has shown a statistically significant 3-5 fold increase in the induction of tumor-specific CD8+ T-cell responses in the Treg-depleted cohort. An ongoing clinical trial, based on preclinical studies in murine models, is evaluating a simpler and potentially superior DC platform which obviates the need to *ex vivo* mature DC. This is of particular interest because we have not observed a beneficial impact of vaccinating patients with non-matured compared to *ex vivo* cytokine-matured DC.

Despite the promising nature of this and other vaccination protocols, stimulating an antitumor immune response is only a first step, and perhaps less critical than thought, within the framework of engendering therapeutically useful immunity in the cancer patient. In the setting of a chronic disease such as cancer it will be also paramount, if not more important, to ensure that the vaccine-induced immune response will be sustained. Enhancing positive costimulatory signaling via 4-1BB, OX40 or CD27, interfering with negative costimulatory

signaling via CTLA-4 or PD-1, or inhibiting the action of negative regulatory products such as SOCS1, GILT, Bax or Bak, represent strategies that can be used to this end.

The prevailing consensus is that tumor growth in the immune competent host is a manifestation of its poor immunogenicity, namely that tumors are not recognized by and do not activate the immune system to a degree that will negatively impact on their growth. This, however, is difficult to reconcile with mounting evidence that tumors across the board have elaborated multiple strategies to suppress immune responses within the tumor environment and/or becoming resistant to it. It is, therefore, difficult to escape the conclusion that such immune evasion mechanisms represent the footprints of an effective antitumor immune response that tumors have elaborated means to circumvent. Developing strategies to counter tumor-induced immune suppression could be, therefore, as or more important than developing potent "vaccination" strategies to induce antitumor immunity. Approaches could include the elimination of immune suppressive subsets such as regulatory T cells, immature myeloid cells, or tumor associated macrophages, or inhibiting the action of immune suppressive mediators which are frequently expressed by tumor cells or its stroma such as TGF $\beta$ , PGE2 or B7H1. What is currently not known, yet key to developing effective strategies, is which are the upstream and critical immune suppressive pathways operating in which tumor.

Murine studies have shown that eliminating CD4+CD25+ regulatory T cells (Tregs) by targeting CD25 with antibodies enhances the immune-mediated rejection of tumors. In a recent clinical trial, partial elimination of CD25 expressing Tregs in renal cancer patients using an IL-2-diphtheria toxin fusion product (ONTAK<sup>®</sup>) led to enhanced vaccine-induced antitumor immunity. Selective and controlled elimination of Tregs, therefore, could represent an important adjunct to cancer immunotherapy. Nevertheless, upregulation of CD25 on conventional antigen-activated T cells poses certain limitations and raises a number of concerns. To date, foxp3, a member of the forkhead/winged-helix family of transcription regulators, is the only product known to be expressed exclusively in Tregs, at least in mice. However foxp3, unlike CD25, is not expressed on the cell surface and hence antibodies or ligand-based reagents cannot be used to eliminate foxp3-expressing cells *in vivo*. One way to eliminate foxp3-expressing Tregs could be through the stimulation of foxp3-specific CTL responses because CD8+ T lymphocytes can recognize antigen derived from any cellular compartment in the form of short 8-10 aa long peptides-MHC class I complexes. Preliminary studies from our laboratory have shown that despite the fact that foxp3 is a self antigen and expressed in the thymus, immunization of mice against foxp3 elicits a robust foxp3-specific CTL response, enhances vaccine-

induced antitumor immunity and, in stark contrast to treatment with  $\alpha$ CD25 Ab, does not interfere with a vaccine-induced antitumor immune response.

In summary, engendering protective immunity in cancer patients will require the development of multi-pronged approaches, both to stimulate potent and sustained antitumor immune responses and to overcome the propensity of tumor cells to evade immune elimination. Increasing evidence suggest that the latter, rather than intrinsic lack of immunogenicity, control the later stages of tumor progression, arguing for increased emphasis on developing means to counter immune suppressive pathways operating in the tumor microenvironment.