

# Evolution of NY-ESO-1 cancer vaccines: Lessons learned

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## Abstract

A rationale for testing the efficacy of active specific immunotherapy is based on recent compelling evidence indicating that increased frequency of CD8+ tumor infiltrating lymphocytes (TILs) is associated with improved survival in several cancers including melanoma, colorectal, breast, prostate, renal-cell, esophageal and ovarian carcinomas. In particular, we recently investigated the relationship between subpopulations of TILs and overall survival in ovarian cancer. We found that patients with higher frequencies of intraepithelial CD8+ T cells demonstrated improved survival compared with patients with lower frequencies (median 55 versus 26 months; Hazard Ratio = 0.33, CI 0.18-0.60,  $P = 0.0003$ ). Subgroups with high versus low intraepithelial CD8+/CD4+ TILs ratios had median survival of 74 and 25 months respectively (Hazard Ratio = 0.30; CI 0.16-0.55;  $P = 0.0001$ ), indicating that CD4+ TILs influence the beneficial effects of CD8+ TILs. This unfavorable effect of CD4+ T cells on prognosis was found to be due to CD25+FOXP3+ Tregs, as indicated by survival of patients with high versus low CD8+/Treg ratios (median 58 versus 23 months; Hazard ratio 0.31, CI 0.17-0.58,  $P = 0.0002$ ). Therefore, the development of strategies to enhance the potential of tumor-antigen specific CD8+ T cells may extend remission rates in ovarian and other malignancies. *These strategies should attempt to prime robust CD8+ T cell expansion, promote durable anti-tumor immunity and counteract the immunosuppressive conditions produced by Tregs in order to reveal greater anti-tumor immunity.*

In this regard, the NY-ESO-1 antigen, initially defined by SEREX in esophageal cancer, has been analyzed extensively in several malignancies. NY-ESO-1 is a member of the cancer/testis antigen family and one of the most spontaneously immunogenic tumor antigens described so far. Frequent expression of NY-ESO-1 has been reported in several tumors including hepatocellular, ovarian, prostate, esophageal, bladder, breast and non-small cell lung carcinomas. Parallel single variable clinical trials assessing NY-ESO-1-based immunogens are currently underway at several institutions around the world under the sponsorship of the Cancer Vaccine Collaborative (CVC, <http://www.cancerresearch.org>). The aim of these early phase trials is primarily to assess the immunogenic potential of different antigenic variants and formulations. The immunogens tested include NY-ESO-1-derived peptides with different adjuvants (e.g. montanide, GMCSF, CHP), recombinant NY-ESO-1 encoding viruses, recombinant proteins formulated with ISCOMATRIX or CHP, and others. The clinical and immunologic results, and lessons from these trials will be summarized.

To illustrate lessons learned from NY-ESO-1 vaccine therapy, two clinical trials in ovarian cancer patients will be presented. The goal of the first clinical trial was to test whether providing

cognate helper CD4+ T cells would enhance the anti-tumor immune response by immunizing with NY-ESO-1 peptide ESO:157-170, that is recognized by HLA-DP4 restricted CD4+ T cells, and HLA-A2 and A24 restricted CD8+ T cells. Specific HLA-DP4-restricted CD4+ T-cell responses were elicited after vaccination with NY-ESO-1<sup>157-170</sup> peptide (emulsified in incomplete Freund's adjuvant) in patients with NY-ESO-1-expressing epithelial ovarian cancer. These vaccine-induced CD4+ T cells were detectable from effector/memory populations without requirement for *in vitro* Treg depletion. However, they were only able to recognize peptide but not naturally processed NY-ESO-1 protein and had much lower avidity compared to NY-ESO-1-specific naive CD4+CD25- T cell precursors or to naturally-occurring CD4+ T cells of cancer patients with NY-ESO-1 antibody. In a second on-going clinical trial, ovarian cancer patients are receiving heterologous prime-boost vaccines consisting of recombinant vaccinia-NY-ESO-1 (rV-NYESO-1) and recombinant fowlpox-NY-ESO-1 (rF-NYESO-1). Lessons from this and other CVC trials will also be discussed.