

# NY-ESO-1 protein-based cancer vaccines: the Melbourne experience

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NY-ESO-1 is a “cancer-testis” (CT) antigen found in a wide variety of malignancies, including 35-45% of melanomas (1, 2). Expression of NY-ESO-1 by tumors commonly leads to spontaneous antibody and T-cell responses. Previous clinical trials involving HLA-A2-restricted NY-ESO-1 peptides have shown that these can be administered safely, generate T-cell responses, and may have clinical benefit (3). However, such approaches are restricted to HLA-A2 positive patients only and rely on the assumption that T-cell responses to this epitope are the most relevant. The use of full-length recombinant protein allows all possible relevant epitopes to be available for both HLA class I- and II-restricted responses.

Previous studies have shown that NY-ESO-1 formulated with specific antibodies as immune complexes (NY-ESO-1/IC) leads to enhanced uptake and processing by DCs (4). We were interested in comparing these functions when full length, recombinant NY-ESO-1 was formulated with ISCOMATRIX™ adjuvant (CSL Limited), a saponin-based adjuvant that has been shown to promote cellular and humoral immune responses in NY-ESO-1 pre-clinical models (5). The mode of antigen delivery was found to be a determining factor for cytosolic proteolysis by DC. NY-ESO-1/IC targeted a slow, proteasome-dependent cross-presentation pathway, whereas NY-ESO-1+ ISCOMATRIX™ adjuvant (NY-ESO-1/IMX) targeted a fast, proteasome-independent pathway. Both cross-presentation pathways resulted in a long-lived T cell stimulatory capacity, which was maintained for several days longer than for DCs pulsed with peptide. This may provide DCs with ample opportunity for sensitizing tumor-specific T cells against a broad array of tumor antigen epitopes in lymph nodes and thus has important implications for vaccine design and immunization strategies.

We performed a clinical trial (LUD99-008) in collaboration with CSL Limited, using NY-ESO-1/IMX. The protein was synthesized at CSL and purified at the Ludwig Institute Biological Production Facility in Melbourne. Forty-six evaluable patients with resected NY-ESO-1 positive tumors received three doses of NY-ESO-1/IMX intramuscularly at monthly intervals (6). The vaccine was well tolerated. We observed high titer antibody responses, strong DTH reactions, and circulating CD8+ and CD4+ T cells specific for a broad range of NY-ESO-1 epitopes, including known and previously unknown epitopes (6, 7). In an unplanned analysis, vaccinated patients appeared to have superior clinical outcomes to those treated with placebo or protein alone.

These observations have led to the initiation of several further clinical trials. In trial LUD2002-013, patients with advanced malignant melanoma whose tumors express NY-ESO-1 are

being treated with NY-ESO-1/IMX. The objectives of this ongoing study are to determine in this patient population with advanced disease whether NY-ESO-1/IMX has anticancer activity, to study the immune responses elicited by vaccination, and to determine the safety of NY-ESO-1/IMX. This trial is currently accruing and a total of 25 patients is planned. Trial LUD2003-009, due to commence late 2004 or early 2005, is a randomized phase II trial in which high risk melanoma patients with resected stage III or IV disease will be treated with NY-ESO-1/IMX or with ISCOMATRIX™ adjuvant alone. This study will determine the relapse-free survival at 18 months using these approaches and will provide data to allow power calculations for a future randomized phase III study that will determine whether NY-ESO-1/IMX vaccination can prevent or delay relapse in this population. A critical aspect of this trial will be a rapid, simple and generalizable assay to detect patients with immune responses.

Based on the data from our previous studies and the observations concerning antigen processing pathways in DCs using NY-ESO-1/IMX, we have initiated trial LUD2003-013. This is a pilot study in which autologous peripheral blood DCs will be harvested, pulsed *ex vivo* with NY-ESO-1/IMX, and administered back to patients with resected NY-ESO-1 positive cancer. The nature of the immune responses elicited will be determined and qualitative comparisons with the previous LUD99-008 trial will be made.

Multivalent vaccines are likely to be necessary ultimately in order for vaccines using defined antigens to be more generally applicable. Other antigens and more simple methods of antigen delivery will need to be investigated. Future work in this program will examine the implications of different methods of antigen delivery to antigen-presenting cells both *in vivo* and *ex vivo*, and also whether the rules for NY-ESO-1 also apply to other antigens. This work will only succeed if appropriate, feasible and relevant immunological and clinical endpoints are included in the study designs and if there is close integration between the laboratory and the clinic.

## References

1. Scanlan MJ, Simpson AJ, Old LJ. The cancer/testis genes: Review, standardization, and commentary. *Cancer Immun* 2004; 4: 1. (PMID: 14738373)

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2. Vaughan HA, Svobodova S, MacGregor D, *et al.* Immunohistochemical and molecular analysis of human melanomas for expression of the human cancer testis (CT) antigens NY-ESO-1 and LAGE-1. *Clin Cancer Res*. In press.
3. Jäger E, Gnjatic S, Nagata Y, Stockert E, Jäger D, Karbach J, Neumann A, Rieckenberg J, Chen YT, Ritter G, Hoffman E, Arand M, Old LJ, Knuth A. Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. *Proc Natl Acad Sci U S A* 2000; **97**: 12198-203. (PMID: 11027314)
4. Nagata Y, Ono S, Matsuo M, Gnjatic S, Valmori D, Ritter G, Garrett W, Old LJ, Mellman I. Differential presentation of a soluble exogenous tumor antigen, NY-ESO-1, by distinct human dendritic cell populations. *Proc Natl Acad Sci U S A* 2002; **99**: 10629-34. (PMID: 12138174)
5. Maraskovsky E, Sjolander S, Drane DP, Schnurr M, Le TT, Mateo L, Luft T, Masterman KA, Tai TY, Chen Q, Green S, Sjolander A, Pearse MJ, Lemonnier FA, Chen W, Cebon J, Suhrbier A. NY-ESO-1 protein formulated in ISCOMATRIX adjuvant is a potent anticancer vaccine inducing both humoral and CD8+ T-cell-mediated immunity and protection against NY-ESO-1+ tumors. *Clin Cancer Res* 2004; **10**: 2879-90. (PMID: 15102697)
6. Davis ID, Chen W, Jackson H, Parente P, Shackleton M, Hopkins W, Chen Q, Dimopoulos N, Luke T, Murphy R, Scott AM, Maraskovsky E, McArthur G, MacGregor D, Sturrock S, Tai TY, Green S, Cuthbertson A, Maher D, Miloradovic L, Mitchell SV, Ritter G, Jungbluth AA, Chen YT, Gnjatic S, Hoffman EW, Old LJ, Cebon JS. Recombinant NY-ESO-1 protein with ISCOMATRIX adjuvant induces broad integrated antibody and CD4+ and CD8+ T-cell responses in humans. *Proc Natl Acad Sci U S A* 2004; **101**: 10697-702. (PMID: 15252201)
7. Chen Q, Jackson H, Parente P, Luke T, Rizkalla M, Tai TY, Zhu HC, Mifsud NA, Dimopoulos N, Masterman KA, Hopkins W, Goldie H, Maraskovsky E, Green S, Miloradovic L, McCluskey J, Old LJ, Davis ID, Cebon J, Chen W. Immunodominant CD4+ responses identified in a patient vaccinated with full-length NY-ESO-1 formulated with ISCOMATRIX adjuvant. *Proc Natl Acad Sci U S A* 2004; **101**: 9363-8. (PMID: 15197261)