

Comprehensive analysis of T-cell responses after vaccination with NY-ESO-1 protein

Weisan Chen^{1*}, Heather Jackson¹, Nektaria Dimopoulos¹, Tsin Yee Tai¹, Nicole A. Mifsud¹, Qiyuan Chen¹, Lena Miloradovic², Eugene Maraskovsky^{1,3}, Lloyd J. Old², Ian D. Davis¹, and Jonathan S. Cebon¹

¹Ludwig Institute for Cancer Research, Melbourne, Australia

²Ludwig Institute for Cancer Research, New York, NY

³CSL Limited, Melbourne, Australia

*Presenting author

Antigen-specific CD8+ T cells can play an important role in the development of cancers through the process of immunoediting (1). Many cancer immunotherapy trials are based upon this rationale. Few clinical trials to date have achieved the ultimate goal of eliminating all cancer cells and cure of the disease. As a result, surrogate measurements are often used for monitoring trial outcomes. In order for an immunotherapy trial to be interpretable it is important that immunological monitoring be well characterized and validated.

Although peptide-based trials are relatively easy to conduct and monitor, overall their efficacy has been disappointing. Full-length tumor antigen based strategies are attracting more attention due to the integration of T cell help, which is essential for both cytotoxic T lymphocyte (CTL) priming and recall phase expansion. Full length protein also provides the opportunity for the host antigen presenting cells to process relevant immunodominant epitopes. However, monitoring of vaccinated T-cell responses remains challenging, particularly if the key epitopes have not been defined, or if there are responses to multiple determinants.

NY-ESO-1 is an immunogenic but relatively small (180 amino acids) "cancer-testis" antigen. We have evaluated cellular immune responses in two patient populations; (i) a series of melanoma patients whose tumors expressed the NY-ESO-1 antigen and in whom spontaneous immune responses developed during the course of their disease, (ii) patients who were vaccinated with recombinant full-length NY-ESO-1 protein produced in a bacterial expression system and formulated with ISCOMATRIX™ adjuvant (NY-ESO-1/IMX) (LUD99-008) in collaboration with CSL Limited (2, 3, 4). Only limited HLA serotyping data were available for the trial participants. Without knowledge of the potential CD8+ and CD4+ T-cell epitopes for each individual patient, we developed a robust method for *in vitro* T-cell expansion and systematically monitored T-cell responses from both vaccinated patients and patients who had shown naturally induced anti-NY-ESO-1 antibody responses. Utilizing multiple sets of synthetic overlapping peptides as well as various sources of full-length NY-ESO-1 protein, very broad T-cell responses to NY-ESO-1 epitopes have been identified, including previously known and unknown ones. We were also able to demonstrate that the majority of the detected CD8+ and CD4+ T-cell responses were induced by vaccination with NY-ESO-1/IMX, because the same responses were not detected from pre-vaccination samples of the same patients. Some NY-ESO-1-specific T cells were primed after the first vaccination and boosted subsequently. We also detected polyclonal CD8+ and CD4+ T-cell responses against multiple epitopes from naturally induced NY-ESO-1-specific responses from some patients with

serum antibody to NY-ESO-1. The NY-ESO-1 sequence 79-110 contains an epitope-rich area for vaccinated patients despite only limited sharing of HLA alleles. Analysis of T-cell responses specific to this region of the NY-ESO-1 antigen might serve as a quick screening strategy for both naturally induced and vaccinated immunity. Under similar monitoring conditions, no NY-ESO-1-specific response was detected for samples collected from normal individuals.

This kind of detailed T-cell analysis will not only help us to further understand the scope, efficacy and the immunodominance hierarchy of both natural and vaccinated anti-tumor cellular immune responses, but also help us to identify the most immunodominant T helper and cytotoxic T-cell epitopes. This in turn may be very important for future vaccine development and more efficient trial monitoring.

References

- Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. *Nat Immunol* 2002; **3**: 991-8. (PMID: 12407406)
- 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, Gnjjatic S, Hoffman EW, Old LJ, Cebon JS. Recombinant NY-ESO-1 protein with IS-COMATRIX 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)
- 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)
- Jackson HM, Dimopoulos N, Chen Q, Luke T, Yee Tai T, Maraskovsky E, Old LJ, Davis ID, Cebon J, Chen W. A robust human T cell culture method suitable for expanding tumor specific CD8+ and CD4+ T cells to known and unknown epitopes. *J Immunol Methods* 2004; **291**: 51-62. (PMID: 15345304)

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