Responses of the patients to vaccine. As well as measurement of IgG antibodies to WT1 following WT1 peptide vaccination were 0.29% and 0.43% respectively. At the same time-point decrease in the tumor markers level was observed. Tetramer and intracellular IFN-γ assays as well as measurement of IgG antibodies to WT1 following WT1ptide-based vaccination give the valuable information for monitoring the immune responses of the patients to vaccine.

His-tag ELISA for the Detection of Humoral Tumor-Specific Immunity

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The enzyme-linked immunosorbant assay (ELISA) method is a powerful, rapid and simple laboratory assay which can be designed to measure proteins (e.g. antibodies) in human sera for the purposes of diagnostics or other biomarker strategies. Typically, antibody ELISAs are done in 96-well plates, which are coated with protein or peptide to capture serum antibodies. A major problem limiting the use of antibody ELISA is the lack of purified proteins that are the targets of the serum antibodies. We hypothesized that mammalian cell expression of a histidine (his)-tagged human protein could be used to produce proteins suitable for detecting serum antibodies. Our goal was to develop a his-tagged capture ELISA for detection of antibodies to insulin-like growth factor binding protein 2 (IGFBP2), a potential tumor antigen overexpressed by tumors. Sensitivity, accuracy, linearity, and precision for each approach met uniform clinical laboratory standards, suggesting that the assay could be validated for diagnostic use. Comparing the his-tag capture ELISA with a standard indirect ELISA revealed that specificity was lower for the his-tag assay (p < 0.008). The level of detection for the indirect ELISA was significantly lower than for the his-tag assay (p < 0.0000), but sample concordance was also highly significant (p = 0.003). These results suggest that ELISAs based on the his-tag approach may be suitable substitutes for standard indirect ELISAs, obviating the need for production and purification of protein antigens.

The Level of HER-2/neu Protein Expression in Primary Breast Cancer May Impact the Development of HER-2/neu Specific Humoral Immunity

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Aberrant expression of tumor antigens results in the generation of immunity. Although the mechanisms involved in the generation of tumor antigen-specific immune responses are unclear, overexpression may result in increased exposure of the immune system to epitopes not ordinarily observed with normal levels of expression. Thus, immunity may increase as the level of expression of the tumor antigen increases. We investigated this hypothesis in HER-2/neu overexpressing cancers by questioning whether the magnitude and incidence of humoral immune response to self-antigen HER-2/neu (HER2) was influenced by the level of HER2 overexpression. We also questioned whether level of HER2 overexpression was associated with the ability to mount a HER2-specific antibody response to tumor vaccine. We compared the HER2 overexpression, as determined by immunohistochemistry (IHC), from 90 women with Stage II, II and IV breast cancer, with the levels of HER2-specific antibodies. HER2-overexpression was categorized into 3 groups, low (n = 16), medium (n = 10), and high (n = 64). As a surrogate marker for immune competence, we measured tetanus toxoid-specific antibodies in 62 of the women. Although there were no significant differences in magnitude or incidence of HER2-specific antibodies between IHC groups (p-values of 0.110 and 0.184, respectively), there was a trend toward an increase in level (p = 0.073) or incidence (p = 0.068) of HER2-specific antibodies as HER2 overexpression increased. Furthermore, there was no relationship between the levels of HER2-specific antibodies and the ability to augment antibody immunity to HER2 following immunization with a HER2 peptide-based vaccine.

Immune Monitoring with iTAG™ MHC Tetramers*: Potential Clinical Utility in the Prediction of Recurrent CMV Reactivation in Allogeneic Stem Cell Transplant Recipients

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This study evaluated the use of tetramers in monitoring CMV-specific T-cell recovery following allogeneic stem cell transplantation (SCT) to predict patients at risk for CMV-related complications. SCT recipients were monitored for up to 944 days for viremia, lymphocyte counts, and clinical status. iTAG MHC Tetramers (Beckman Coulter, San Diego) were used to enumerate CMV-specific CD8 T-cells by flow cytometry using a single-platform absolute counting method. The following tetramers were included: pp50: A*0101 (VTEHDLTLY), pp65: A*0101 (YSEEHFTTQSY), A*0201 (NLVPMAVTV), A*2402 (QYDPVAAFL), B*0702 (TPRTVGGGAM), B*5301 (IPSINHHYH); pp150: A*0301 (TVYPSSSTAK); IE-1: A*0201 (VLEETSVML), B*0801 (ELRRKMMTYM). Data were analyzed for 36 myeloablative CMV-seropositive patients with early tetramer values available (0–100 days). Reduced intensity regimens will be analyzed separately when adequate follow-up times have been obtained. Study results indicate that myeloablated patients with a slow rate of recovery of CMV-specific T-cells (able to mount a response above 2 cells/μl in the first 100 days post transplant) were at risk for complications. Patients with a maximum response <2 cells/μl were 3.8 times more likely to have recurrent viremia (p = 0.01). Of interest, this patient group was also 2.5 times more likely to develop extensive cGVHD (p = 0.03), and 3 times more likely to develop non-relapse fatal complications (p = 0.004). Tetramers have potential clinical utility in monitoring CMV-specific T-cells to predict patients at risk of CMV-related complications, allowing clinicians to further refine pre-emptive therapeutic strategies in appropriate high-risk populations, and possibly in the future to monitor pre- and post- antigen-specific cellular immunotherapy. *For research use only. Not for use in diagnostic procedures.

Detection of Influenza Virus Specific CD4+ T Cells Using a Panel of MHC Class II, DR1, DR3, and DR4 Tetramers

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The identification and enumeration of antigen specific CD4+ T helper cells in the peripheral blood provides a means for monitoring the immune response of tumor antigens. The peripheral blood provides a means for monitoring the immune response of tumor antigens.