Extended Immunological Analysis of Two Phase 1 Clinical Trials of MVA-BN®-HER2 in HER-2 Overexpressing Metastatic Breast Cancer Patients

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Abstract

MVA-BN®-HER2 is a poxviral vector that encodes the extracellular domain of human HER-2 as well as two universal tetanus T cell epitopes. Preclinical data have demonstrated MVA-BN®-HER2 to be immunogenic, inducing strong antitumor activity against HER-2 expressing tumors (Pineda et al., 2008). Previous immunological evaluation of MVA-BN®-HER2 treated patient samples revealed that treatment was able to break tolerance against HER-2 in a metastatic setting, inducing a humoral and/or T-cell response in greater than 66% of the patients. Specifically, anti-HER-2 antibodies were detected in 52% of patients tested and T-cell responses were boosted in 63% of patients (Reported at the 36th Annual CTRC-AACR San Antonio Breast Cancer Symposium. Abstract #2247). Here we report on extended immunological analysis of cryopreserved PBMCs and sera from patients receiving MVA-BN®-HER2. The MVA-BN® viral vector activated innate immune responses, potentially propagating antitumor responses. This was noted by the detection of natural killer cytolytic activity in 50% of evaluated patients as well as measurement of gamma-delta T cells, a population having direct antitumor cytolytic functions. Adaptive cellular immune responses were also evident post treatment. MVA-BN®-HER2 vaccination elevated CD8 effector T cell levels, resulting in an increased CD8 effector to Treg ratio. In contrast, high levels of CD4/CD8 double positive T cell levels, a possible regulatory population, were detected in low responding patients.

Humoral immune responses were further analyzed in two new assays: (1) a flow cytometry based tier assay to characterize anti-HER-2 antibody binding to HER-2 expressing cells, and (2) a peptide array comprised of 7590 peptides derived from breast cancer tumor associated antigens (TAAs) including HER-2. In patients treated with MVA-BN®-HER2, qualitatively different anti-HER-2 antibody responses were measured by these assays as compared to previous ELISAs. In addition, the peptide array assay revealed that repeated treatment was accompanied by a broadening of the anti-HER-2 humoral response as well as epitope spreading to other TAAs.

Treated patient sera revealed that treatment was able to break tolerance against HER-2 in a metastatic setting, inducing a humoral and/or T-cell response in greater than 66% of the patients.

Results

MVA-BN®-HER2 Clinical Trial Observations

Immunohistopathtic Analysis of Patients Treated with MVA-BN®-HER2

Detection of HER-2 Specific Antibody Responses with a Cell-Based Flow Cytometry Assay

We performed extended immune monitoring of patients receiving MVA-BN®-HER2 that had previously been evaluated for cell-based responses by ELISPOT and ELISA (Poster #5588, 37th Annual CTRC-AACR San Antonio Breast Cancer Symposium. Abstract #2247). The current study established the suitability of additional immunological assays for the measurement of innate, adaptive cellular and humoral arms of the immune response.

Innate Immune Responses

- Natural killer (NK) cytolytic activity was detected in 50% of evaluated patients. The assay was established as a suitable method for the detection of NK responses in clinical patients.
- Immunophenotyping revealed
  - Low γδ T-cell levels in two patients (07-057 and 07-077) showing stable disease
  - High levels of CD4/CD8 double positive T cells, a potential population regulating T-cells, were detected in patients who lacked a treatment induced boost of HER-2 antibody responses (07-058, 08-003, and 08-030)

Adaptive Cellular Immune Responses

- Immunophenotyping revealed
  - Elevated CD8 effector T cell levels (data not shown) and lack of a treatment induced effect on Treg's, resulting in an increased CD8 effector to Treg ratio
  - Increased CD107 expression by CD8 T-cells in two stable patients (07-057 and 07-077), which correlated inversely with γδ T-cell levels
  - Co-expression of CD54/CD95 by CD8 T-cells as a possible biomarker of antigen-specific activation
  - Evidence of CD4 T-cell exhaustion by PD-1 upregulation

Humoral Immune Responses

- A flow cytometry based assay to characterize anti-HER-2 antibody binding to HER-2 expressing cells revealed responses in 2 patients with undetectable anti-HER-2 IgG ELISA levels.

The JPT RepliTope™ peptide microarrays, comprised of 7590 peptides derived from 46 breast cancer tumor associated antigens (TAAs) including HER-2, revealed a broadening of the anti-HER-2 humoral response as well as epitope spreading to other TAAs.

In general, variability in detectable responses was evident across the patients.

Specific antigens did not appear to be recognized in common; however, as expected, the highest frequency responses were to breast cancer associated antigens.

Qualitatively different anti-HER-2 antibody responses were measured by these assays as compared to previous ELISAs.

Summary

Detection of NK Cell Innate Immune Responses in 4 out of 8 Patients Treated with MVA-BN®-HER2

Luciferase-Based NK Cell Cytotoxicity Assay

A K562-Luciferase target cell line was generated. NK cells were isolated from patient PBMCs using magnetic based negative selection. NK effector cells were co-cultured with Target K562-Lucerase cells for 4 hours at various effector to target ratios. Percent cytotoxicity was calculated as the experimental luminescence minus spontaneous luminescence divided by maximum luminescence minus spontaneous luminescence. Maximal spontaneous luminescence was assessed by complete lysis of target cells with 1% Nonidet P-40. Boosted NK cell cytotoxicity was measured post treatment in 4 out of 8 patients.

Detection of Serum Antibodies to TAAs with RepliTope™ Peptide Microarrays

Use of RepliTope™ technology to analyze multiple patients receiving MVA-BN®-HER2 treatment provides a comprehensive indication of immunodominance to each number of TAAs to which each patient had a response. Induced (designated by the symbol +) and (designated by the symbol -) TAA response to peptides across all proteins are shown. An induced/lost response ratio was also calculated for each patient. The loss of detectable responses may be associated with an immune escape or increased protein expression levels as a result of therapy. A total of 11 peptides from 5 TAA were induced in 4 patients. None of the patients tested had a detectable response to the remaining 26 peptides.