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 toxin T cell epitopes. Preclinical data have demonstrated MVA-BN®-HER2 to be immunogenic, inducing strong antitumor activity against HER-2 expressing tumors *(iSBTc 2010 Poster #113, Mandl et al.)*. 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 32nd Annual CTRC-AACR San Antonio Breast Cancer Symposium, Abstract #5089, Legrand et al.*).

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 cytotoxic 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 titer assay to characterize anti-HER-2 antibody binding to HER-2 expressing cells, and (2) a peptide array comprised of 7590 peptides derived from 46 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.

Taken together, these data support that MVA-BN®-HER2 treatment is an activator of both the innate and adaptive arms of the immune response. The broadening of immune responses to non-HER-2 TAAs suggests that the MVA-BN®-HER2-mediated immune

Summary

We performed extended immune monitoring of patients receiving MVA-BN®-HER2 that had previously been evaluated for T-cell and antibody responses by ELISPOT and ELISA (*Poster #5089, 32nd Annual CTRC-AACR San Antonio Breast Cancer Symposium, Legrand et al.*). **The current studies 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 B-cells, were detected in patients who lacked a treatment induced boost of HER-2 antibody responses (07-058, 08-003, and 08-033)

Adaptive Cellular Immune Responses

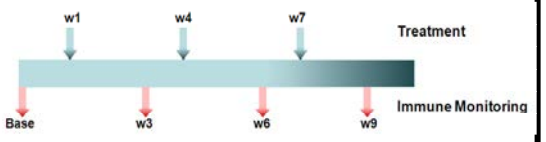
- Immunophenotyping revealed
  - Elevated CD8 effector T cell levels (data not shown) and lack of a treatment induced effect on Tregs, resulting in an increased CD8 effector to Treg ratio
  - Increased CD131 expression by CD8 T-cells in two stable patients (07-057 and 07-077), which correlated inversely with γδ T-cell levels
  - Co-expression of CD54/CD95 by CD8 T-cells as a possible biomarker of antigen-specific activation
  - Evidence of CD8 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 titers.
- 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 measurable by these assays as compared to previous ELISAs.

Study Design

BR-001 and BR-002 MVA-BN®-HER2 Clinical Trial Timeline



Schematic diagram of the MVA-BN®-HER2 clinical trial timeline. *Clinical samples were collected from 30 patients enrolled in two fixed-dose single arm phase I trials under the BNIT BR-001 and BR-002 protocols of IND13211. These trials evaluated MVA-BN®-HER2 in women with metastatic breast cancer. Study BR-001 evaluated patients following first or second line chemotherapy either alone (cohort 1), or in combination with single agent taxane chemotherapy (cohort 2). Study BR-002 evaluated patients following first or second line chemotherapy. Patients were treated subcutaneously with 1×10⁸ $TCID_{50}$ of MVA-BN®-HER2, 3 times at 3 week intervals. All patients from BR-001 and BR-002 were allowed to receive concurrent standard Herceptin treatment, which was administered weekly.*

Results

MVA-BN®-HER2 Clinical Trial Observations

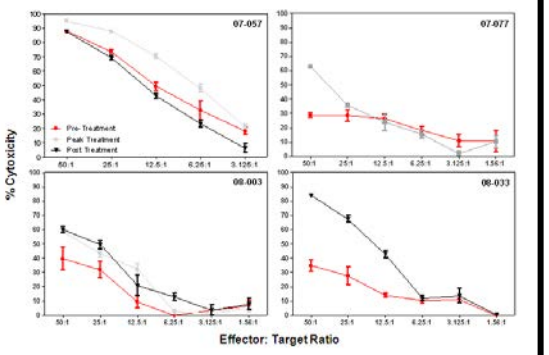
Table 1. MVA-BN®-HER2 Patient Demographics and Clinical Observations.

Study	Cohort	Patient No.	Age	Herceptin Status	Frequency of Peak HER-2 IFNγ ELISPOT Response	Peak HER-2 Specific Humoral Titer	Clinical Status
BR-002	1	07-029	83	Pos	1/6650	1/160	Clinical Progression, Death
		07-033	61	Pos	1/13300	1/320	Clinical Progression
		07-057	63	Pos	1/50000*	1/2560*	Stable
		07-058	50	Pos	1/1330*	1/160**	Clinical Progression
		07-077	51	Pos	1/28500	1/160*	Stable
		08-003	64	Pos	1/1720^A	1/80**	Clinical Progression
		08-005	46	Pos	1/12500	<1/40	Clinical Progression
		08-024	49	Pos	ND	<1/40	Clinical Progression
		08-033	43	Pos	1/5400	<1/40	Clinical Progression
BR-001	1	07-061	60	Pos	ND	1/160	Clinical Progression
		07-063	34	Pos	ND	1/320**	Clinical Progression
		07-064	54	Pos	ND	1/320	Stable
		07-065	44	Pos	ND	1/1280**	Stable
		07-066	63	Pos	ND	ND	Clinical Progression, Death
		07-067	58	Pos	ND	1/80	Stable
		07-071	64	Pos	ND	<1/40	Clinical Progression
		07-072	46	Pos	ND	1/640	Clinical Progression
		08-014	51	Pos	ND	1/320	Stable
		08-018	54	Pos	ND	<1/40	Clinical Progression, Death
		08-029	47	Pos	ND	<1/40	Clinical Progression
	2	07-062	73	Neg	ND	1/640**	Clinical Progression, Death
		08-006	54	Neg	ND	<1/40	Clinical Progression, Death
		08-007	55	Neg	ND	1/1280	Clinical Progression, Death
		08-015	58	Pos	ND	<1/40	Clinical Progression, Death
		08-017	51	Pos	ND	1/40	Stable
		08-023	37	Neg	ND	<1/40	Stable
		08-027	51	Neg	ND	<1/40	Clinical Progression, Death
		08-034	50	Pos	ND	1/1280	Clinical Progression
		08-053	47	Pos	ND	1/1280	Death
		08-055	42	Pos	ND	<1/40	Clinical Progression

* Preexisting Response
**No Change from Baseline
^ HER-2 ICD response was detected
ND, Not Determined

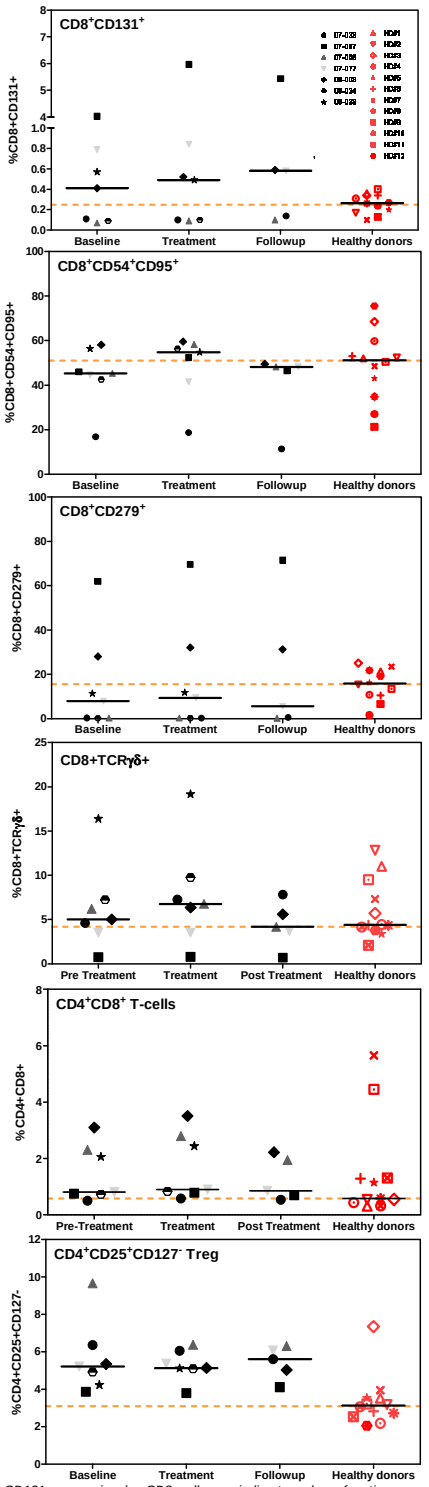
MVA-BN®-HER2 *Clinical Patient Characteristics.* *Previously reported positive HER-2 specific humoral and cell-mediated responses are highlighted in red.*

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



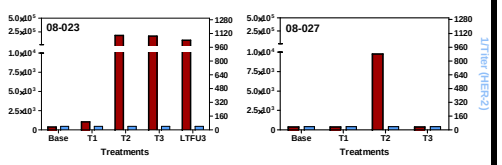
Luciferase-Based NK Cell Cytotoxicity Assay. *A K562-Luciferase target cell line was generated. NK cells were isolated from patient PBMCs using magnetic based negative selection. NK effector cells were co-cultured with Target K562-Luciferase cells for 4 hours at various effector to target ratios. Percent 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.*

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



High CD131 expression by CD8 cells, an indirect marker of antigen specificity was detected in two patients with stable disease (07-057 and 07-077). Upregulation of ICAM-1 (CD54) and FAS-L (CD95), a marker of IFN-induced T-cell activation, as well as evidence of exhaustion (CD279, PD-1) was observed in some patients. γδ T-cells, an early source of IFN-γ having antitumor cytotoxicity, were evident in MVA-BN®-HER2 treated patients. Low levels of γδ T-cells were measured in two patients with stable disease (07-057 and 07-077) having high levels of CD8/CD131 expression. In a subpopulation of patients, high $CD4^+CD8^+$ T cells, characterized by an activated effector/memory CD8 T cell phenotype, cytotoxic potential, as well as a high production of IL-5 and IL-13, which may play a regulatory role, were observed. A higher frequency of $CD4^+CD25^+CD127^-$ Tregs were detected in breast cancer patients as compared to healthy controls. No treatment induced effect on Tregs was evident.

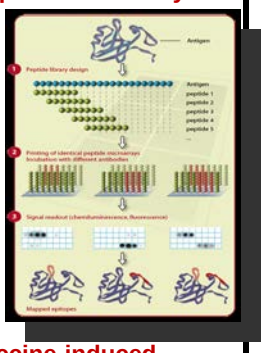
Detection of HER-2 Specific Antibody Responses with a Cell-Based Flow Cytometry Assay



Identification of anti-HER-2 antibody responses in 2 patients with undetectable ELISA IgG titers. *SKBR3 cells were co-incubated with serial diluted patient sera for 30 minutes. Cells were washed and stained with a fluorescent labeled anti-Ig-PE secondary antibody for an additional 30 minutes. Data interpretation is based on the integrated MFI (iMFI), which is the percentage of PE-positive cells multiplied by the mean fluorescence intensity (MFI).*

JPT RepliTope™ Peptide Microarrays

JPT RepliTope™ high density microarrays, provided by JPT Peptide Technologies (Berlin, Germany), were employed to monitor humoral immune responses in patients treated with MVA-BN®-HER2. The RepliTope™ arrays are a novel tool incorporating peptides that are covalently bound to glass slides by non-selective immobilization chemistry using the amino-function of lysine side chains. The array peptides represent a linear scan of 15mer peptides derived from 46 human tumor associated antigens (TAA) including HER-2, constituting a total of 7590 peptides. Human IgG and Herceptin were utilized as controls. Baseline, peak treatment, and if applicable post treatment serum samples from 13 patients were tested.



Detection of Vaccine-induced HER-2 ECD Antibodies with RepliTope™ Peptide Microarrays

Patient ID.	RepliTope Her-2 Response (Peptide Number)	Anti-HER-2 IgG Titers
08-007	373, 377, 489, 549, **605, 609, 613**	1/1280
08-053	381, 385, 597, 601, **605, 609, 613**	1/1280
07-062	373, 377, 381	1/640
07-029	**605, 609**	1/160
07-033	None Detected	1/320
07-072	361, **605, 609, 613**	1/640
07-061	None Detected	1/160
08-029	**605, 609, 613**	<1/40
08-024	385	<1/40
08-034	**605, 609, 613**	1/1280
08-014	None Detected	1/320
07-064	**605, 609, 613**	1/320
07-077	41, 525, 549, 601, **605**	1/160

HER-2 Immunodominant regions (highlighted in red) were identified using the RepliTope™ microarray. *7 patients responded to domain IV of HER-2 ECD. HER-2 responses identified by the microarray technology had a 60% correlation to ELISA data.*

Detection of Serum Antibodies to TAAs with RepliTope™ Peptide Microarrays

Patient ID.	Clinical Status	Total Response # Proteins (# peptides)	Ratio (Induced/Reduced Response)
08-007	Clinical Progression, Death	16 (13+,8+,16-)	0.50
08-053	Death	28 (33+,15+,37-)	0.41
07-062	Clinical Progression, Death	25 (4+,7+,33-)	0.21
07-029	Clinical Progression, Death	21 (27+,5+,32-)	0.16
07-033	Clinical Progression	28 (59+,19+,11-)	1.73
07-072	Clinical Progression	13 (14+,38+,0-)	38.00
07-061	Clinical Progression	2 (2+,0+,0-)	0.00
08-029	Clinical Progression	16 (13+,8+,9-)	0.89
08-024	Clinical Progression	20 (12+,5+,17-)	0.29
08-034	Clinical Progression	12 (14+,8+,8-)	1.00
08-014	Stable	9 (5+,4+,3-)	1.33
07-064	Stable	8 (7+,15+,7-)	2.14
07-077	Stable	26 (13+,28+,36-)	0.27

Use of RepliTope™ technology to identify epitope spreading to TAAs in MVA-BN®-HER2 treated patient sera. *The data are organized by the total number of proteins to which each patient had a response. Induced (designated by the symbol ⁺), lost (designated by the symbol ⁻) or unchanged (designated by the symbol ⁼) responses 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 attributed to immunological escape or decreased protein expression levels as a result of therapy. A total of 11 peptides from 5 TAA to which multiple patients (>4) had a response were identified, perhaps an indicator of immunodominance.*