Evaluation of HER-2 Specific Humoral Immune Responses in Breast Cancer Patients Treated with MVA-BN®-HER2

Fatema Legrand[1], Rachel Owen[1], Amanda Enstrom[1], Olivia Hwang[1], Gayatri Paranjpe[1], Joy Su[1], Bernadette Callejo[1], Alex Chung[1], Jess Nolin[1], Angela Yu[1], Belma Halilovic[1], Olga Bandman[1], Carsten Goessl[1], Ulf Reimer[2], Holger Wenschuh[2], Reiner Laus[1], Wayne Godfrey[1], Alain Delcayre[1]

[1]BN ImmunoTherapeutics, Inc., 2425 Garcia Ave, Mountain View, CA 94043, USA

[2]JPT Peptide Technologies GmbH, Volmerstrasse 5 (UTZ), 12489 Berlin, Germany

fatema.legrand@bn-it.com, alain.delcayre@bn-it.com

BN ImmunoTherapeutics

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 anti-tumor activity (Mandl et al., iSBTC 2010). MVA-BN®-HER2 has also been evaluated in various phase I safety and immunogenicity trials, with 30 HER-2-positive breast cancer patients being tested in the metastatic setting and 15 patients following adjuvant therapy. Previous immunological monitoring of MVA-BN®-HER2 treated patient samples revealed that treatment was able to break tolerance against HER-2 in the adjuvant and metastatic settings, inducing humoral and/or T-cell responses in the majority of the patients (Legrand et al., iSBTC 2010 and Owen et al., SITC 2011).

Extended analysis of humoral responses was performed in patients receiving MVA-BN®-HER2 to determine the relevant immune parameters that correlate with clinical benefit. The generation of HER-2 transgene and MVA vector specific antibody responses was assessed with the ELISA IgG titer assay. The breadth of the anti-tumor response was determined using a peptide array comprised of 7590 peptides derived from 46 breast cancer tumor associated antigens (TAA) including HER-2. In addition, the role of vaccine induced HER-2 specific antibodies in eliciting functional anti-tumor activity is being evaluated.

Overall, it was observed that qualitatively different anti-HER-2 antibody responses were induced in patients treated with MVA-BN®-HER2. 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. Strong responses to 15 TAA proteins were detected in at least 12 out of the 30 tested patients. In addition, 42 out of the 7590 total evaluated peptides were identified as being immunodominant. Importantly, the presence of a pre-existing immune response to the MVA vector did not impair the induction of transgene specific immune responses. The broadening of immune responses to non-HER-2 TAAs suggests that the MVA-BN®-HER2-mediated immune activation results in anti-tumor activity. Taken together, these data support MVA-BN®-HER2 treatment to be a potent activator of humoral immune responses in both the metastatic and adjuvant settings.

Summary

Anti-HER-2 IgG ELISA titers were induced both in the adjuvant (BR-003 clinical trial) and metastatic setting (BR-001/BR-002 clinical trial).

- 13 out of 15 adjuvant patients induced a HER-2 specific IgG titer. Of these patients, only one showed evidence of pre-existing HER-2 responses.
- 15 out of 29 metastatic patients induced a HER-2 specific IgG titer. Of these patients, seven had pre-existing responses (data not shown).

Anti-MVA IgG ELISA titers were also induced or boosted both in the adjuvant (BR-003 clinical trial) and metastatic setting (BR-001/BR-002 clinical trial).

- All 15 adjuvant patients induced a specific IgG response to the MVA vector. Of these patients, eight had pre-existing responses.
- 27 out of 30 metastatic patients induced a MVA specific IgG titer (data not shown), nine of whom had pre-existing responses (data not shown).

The higher frequency of HER-2 and MVA specific humoral responses in the adjuvant setting (87% and 100% response rate, respectively) as compared to the metastatic setting (52% and 90% response rate, respectively) may be attributed to the patients' disease-free status as well as earlier and higher HER2 expression under the synthetic Ps promoter in the second generation MVA-BN®-HER2 vector.

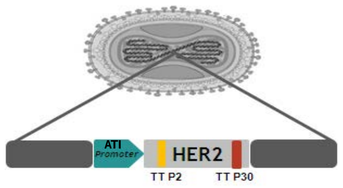
- The JPT RepliTope™ peptide microarrays, comprised of 7590 peptides derived from 46 breast cancer tumor associated antigens (TAAs) including HER-2, revealed a broadening of the anti-HER-2 humoral response as well as epitope spreading to other TAAs.
  - In general, variability in detectable responses was evident across the patients.
  - Strong responses to 15 TAA proteins were detected in at least 12 out of the 30 tested patients. In addition, 42 out of the 7590 total evaluated peptides were identified as being immunodominant.
  - As expected, the highest frequency responses were to breast cancer associated antigens.

MVA-BN®-HER2 Based Cancer Immunotherapies Clinical Trial Characteristics

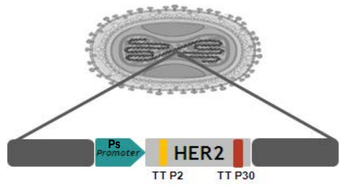
	BR-001/BR-002	BR-003
	Phase I	Phase I
Setting	Metastatic Breast Cancer	Post Adjuvant Breast Cancer
Location	Eastern Europe, US	US
No. of Cohorts/ Type	2/ Concurrent or Post Chemo	1
Treatment Schedule	1E8 $TCID_{50}$ q3wX3	1E8 $TCID_{50}$ q4wX6
Immune Monitoring Time-points	Base, 3 Post Treatment, 2 LTFU	Base, 2 Post Treatment, 2 LTFU
Sample Shipment	Overnight/Same Day Courier	Same Day Courier
Median Age	54 years (range, 34 - 64)	53 years (range, 32 - 67)
Patients Receiving Treatment	30	15
Patients Completing Treatment	29 (3 injections)	15 (6 Injections)
Patients Eligible for Immune Evaluation	29	13
Patients Receiving Concurrent Herceptin Treatment	24	0
Patients Extending Treatment	NA	NA
Drug Related Serious Adverse Events	None	None

Evaluated MVA-BN®-HER2 Vectors

BR-001/BR-002
Metastatic Setting
ATI Promoter



BR-003
Adjuvant Setting
Ps promoter



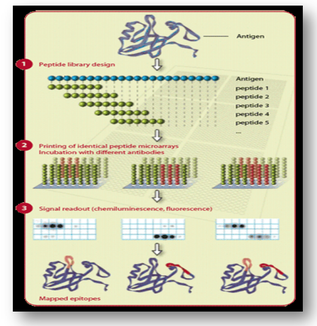
Vaccine-induced HER-2 Specific IgG Titers in MVA-BN®-HER2 Treated Adjuvant (BR-003) Patients

Patient No.	BNIT Study No.	Age	Timepoint	HER-2	MVA
1	10-096	67	Base	<100	3200-6400
			Peak	443	15026
			LTFU	<100	14575
2	10-097	56	Base	<100	<200
			Peak	2969	12739
			LTFU	7939	16555
3	10-099	67	Base	5775	<200
			Peak	2150	124630
			LTFU	100-200	57357
4	10-120	43	Base	<100	<200
			Peak	14065	50605
			LTFU	1021	10590
5	10-126	53	Base	<100	1871
			Peak	1030	8067
			LTFU	200-400	7467
6	10-130	66	Base	<100	<200
			Peak	971	8163
			LTFU	200-400	6378
7	10-145	65	Base	<100	<200
			Peak	200-400	24999
			LTFU	100-200	11469
8	10-174	47	Base	<100	3735
			Peak	3129	7835
			LTFU	<100	3963
9	10-178	35	Base	<100	<200
			Peak	958	14636
			LTFU	NA	NA
10	10-179	66	Base	<100	3521
			Peak	100-200	14773
			LTFU	<100	11822
11	11-021	41	Base	<100	5397
			Peak	1992	28513
			LTFU	200-400	15083
12	11-022	49	Base	<100	2018
			Peak	6282	61958
			LTFU	1028	30013
13	11-056	47	Base	<100	1875
			Peak	18509	57456
			LTFU	NA	NA
14	11-057	32	Base	<100	<200
			Peak	7904	29187
			LTFU	100-200	932
15	11-093	55	Base	<100	1733
			Peak	1950	7305
			LTFU	<100	3861

Sera from prior to, during and long-term follow-up (LTFU) treatment time-points were utilized for HER-2 and MVA IgG antibody titer determination by ELISA. The values represented are the peak titers. Vaccine-induced responses are indicated in red. Vaccine-induced anti-HER-2 antibodies were detected in 13 out of 15 patients tested. One subject (10-099) had pre-existing HER-2 specific titers and did not induce titer above the pre-existing value. The second subject (10-179) neither had a pre-existing titer to HER-2, nor induced a titer. Vaccine-induced anti-MVA antibodies were induced or boosted in all 15 patients. Eight of these subjects had pre-existing anti-MVA IgG titers. Pre-existing titers did not impede the induction of transgene specific responses.

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 30 patients were tested.



Detection of Vaccine-induced HER-2 ECD Specific Antibodies with RepliTope™ Peptide Microarrays in Metastatic (BR-001/BR-002) Patients

Study	Patient	Clinical Status	# Her-2 Peptide Responses	Peak anti-HER-2 Titer
BR-002	07-029	Death	2	1/160
	07-033	Progr.	ND	1/320
	07-033*		ND	
	07-057	Stable	6	1/2560
	07-058	Progr.	12	1/160
	07-077	Stable	42	1/160
	07-077*		5	
	08-003	Progr.	ND	1/80
	08-005	Progr.	1	<1/40
	08-024	Progr.	2	<1/40
	08-024*		1	
	08-033	Progr.	5	<1/40
BR-001/Cohort 1	07-061	Progr.	ND	1/160
	07-063	Progr.	ND	1/320
	07-064	Stable	3	1/320
	07-065	Stable	5	1/1280
	07-066	Death	17	ND
	07-067	Stable	5	1/80
	07-071	Progr.	4	<1/40
	07-072	Progr.	4	1/640
	08-014	Stable	ND	1/320
	08-018	Death	1	<1/40
	08-029	Progr.	3	<1/40
BR-001/Cohort 2	07-062	Death	3	1/640
	08-006	Death	74	<1/40
	08-007	Death	7	1/1280
	08-015	Death	30	<1/40
	08-017	Stable	7	1/40
	08-034	Progr.	3	1/1280
	08-053	Death	7	1/1280

Previously Reported

* Repeated

Responses to HER-2 ECD peptides were identified per patient using the RepliTope™ microarray. *22 patients responded to domain IV of HER-2 ECD. HER-2 responses identified by the microarray technology had a 60% correlation rate to ELISA data.*

Detection of Vaccine-induced Serum Antibodies to TAAs with RepliTope™ Peptide Microarrays in Metastatic (BR-001/BR-002) Patients

	Tumor Associated Antigen (TAA)	# Peptides	# Patients 18 Total	Immunodominant Peptide/s
BRCA-1	Breast cancer type 1 susceptibility protein	69	12	BRCA_P38398_0301(9), BRCA_P38398_1501(9)
BRCA-2*	Breast cancer type 2 susceptibility protein	194	15	BRCA_P51587_0129(14)
HER-1 (EGFR)*	Epidermal growth factor receptor	70	13	HER_P00533_0285(12), HER_P00533_0549(11)
HER-2 *	Receptor tyrosine-protein kinase erbB2	100	14	Her_P04626_0477(13), Her_P04626_0601(13)
HER-3	Receptor tyrosine-protein kinase erbB-3	122	13	HER_P21860_0577(13), HER_P21860_0825(11)
HER-4	Receptor tyrosine-protein kinase erbB-4	93	14	HER_Q15303_0161(15), HER_Q15303_0625(15), HER_Q15303_0637(17)
Mammoglobin	Secretoglobin family 2A member 2	2	2	Mammoglobin_Q13296_0013/77(1)
Lactadherin	Milk fat globule-EGF factor 8	15	4	Lactadherin_Q08431_0045/105/165/257/361/(3),
Muc1 (CA 15-3)	Mucin-1; Breast carcinoma-associated antigen DF3	79	8	Muc1(CA15_P15941_1205(17)
CEA	Carcinoembryonic antigen-related cell adhesion	51	10	CEA_P06731_0001(18)
5T4	5T4 oncofetal trophoblast glycoprotein	18	9	5T4_Q13641_0229(6)
AKT2*	RAC-beta serine/threonine-protein kinase	39	12	AKT2_P31751_0321/329/341(14/15)
ANGPT-1	Angiopoietin-1	20	7	ANGPT_Q15389_0449(11)
ATP6S1	V-type proton ATPase subunit S1	48	16	ATP6S1_Q15904_0433/381(27/25)
Brachyury	Transcription factor	26	8	Brachyury_O15178_0373(15)
c-Myb	Transcriptional activator	46	13	c_P10242_0081/181/317(10)
c-Myc	Myc proto-oncogene protein	9	6	c_P01106_0021(3)
Cyclin B1	G2/mitotic-specific protein	47	13	CyclinB1_P14635_0257(10)
flk-1 (VEGFR-2)	Vascular endothelial growth factor receptor 2; Protein-tyrosine kinase receptor	55	14	flk_P35968_0053/445 (13)
gp96	Tumor rejection antigen/endoplasmic reticular heat shock protein gp96 homolog protein	24	9	gp96_P14625_0329(15)
Histone H4 peptide	Core component of nucleosome octamer	2	2	HistoneH4peptide_P62805_0013(2)
Ip-10	C-X-C motif chemokine 10	1	2	Ip_P02778_0073(4)
MAGE-A3	Melanoma-associated antigen 3	22	11	MageA3_P43357_0293(12)
Mesothelin	Pre-pro-megakaryocyte-potentiating factor	16	5	Mesothelin_Q13421_0101(3)
MICA	MHC class I polypeptide-related sequence A	10	5	MICA_Q29983_0053(3)
MIF	Macrophage migration inhibitory factor	2	2	MIF_P14174_0025/33 (2)
ML-1Ap (BIRC7)	Melanoma inhibitor of apoptosis protein	13	6	ML_Q96CA5_0113(8)
NYBR1*	Ankyrin repeat domain-containing protein 30A	141	12	NYBR1_Q9BXX3_1329(14)
NY-ESO-1	Cancer/testis antigen 1	5	5	NY_P78358_0069(12)
Progranulin	Granulins, Paragranulin	61	9	Progranulin_P28799_0525(12)
SLPI	Antileukoproteinase	9	8	SLPI_P03973_0033(8)
SOX2	Transcription factor	15	9	SOX2_P48431_0153(13)
Survivin (BIRC5)	Apoptosis inhibitor	22	11	Survivin(BIRC5)_O15392_0005(13_)
TERT (I540 peptide)	Telomerase reverse transcriptase	144	14	TERT(I540peptide)_O14746_0165(15)
TRAIL Receptor-2 (DR5)	Tumor necrosis factor receptor superfamily member 10B	12	7	TRAILReceptor_O14763_0237(9)
VEGF-A	Vascular endothelial growth factor A	50	14	VEGF_P15692_0169(21)

Use of RepliTope™ technology to identify epitope spreading to TAAs in MVA-BN®-HER2 (BR-001/BR-002) treated metastatic patient sera. The data are organized by the total number of proteins and individual immunodominant peptides to which patients had a response. Proteins with responses by multiple patients (>12) are highlighted in yellow.