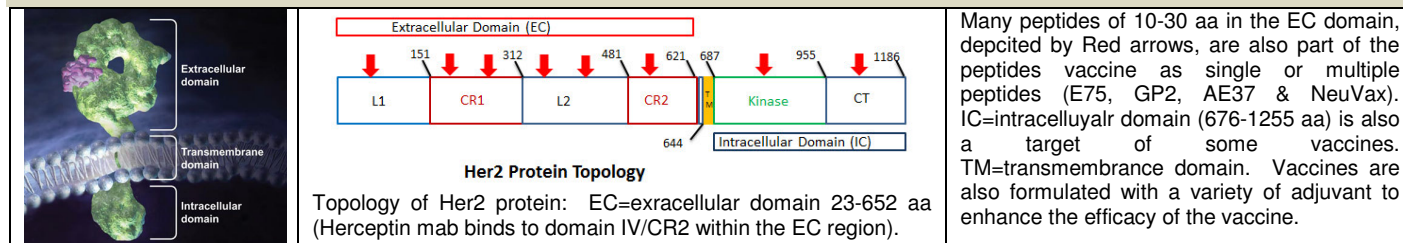


Breast cancer is a type of cancer originating from breast tissue of humans and other mammals. Worldwide, breast cancer comprises 23% of all cancers in women. In 2008, breast cancer caused 458,503 deaths worldwide (13.7% of cancer deaths in women). Breast cancer is more than 100 times more common in women than in men. Prognosis and survival rates for breast cancer vary greatly depending on the cancer type, stage, treatment, and geographical location of the patient. Self-examination, mammography and clinical breast exam can indicate an approximate likelihood that a lump is cancer. Breast cancers are classified by several grading systems (histopathology, Grade, Stage, Receptor status such as ER/PR/Her2 positive). Each of these influences the prognosis and can affect treatment response. Breast cancer is usually treated with surgery and then possibly with chemotherapy or radiation, or both. A multidisciplinary approach is preferable. For the purpose of “**Breast Cancer Vaccine**”, we will discuss “Her2 positive” cancers that comprise about 30% of breast cancer.



HER2 (Human Epidermal Growth Factor Receptor 2) also known as Neu, ErbB-2, CD340 (cluster of differentiation 340) or p185 is a protein that in humans is encoded by the ERBB2 gene. HER2 is a member EGFR/ErbB family. The HER proteins, including Her2, regulate cell growth, survival, adhesion, migration, and differentiation—functions that are amplified or weakened in cancer cells. Since breast cancer cell overexpress and need Her2 protein for their proliferation, a direct or indirect neutralization of Her2 should impair the ability of breast cancer to spread and grow. **Herceptin** (trastuzumab made by Genentech and approved in 1998) is a humanized monoclonal antibody that binds to Her2 protein and interferes with its functions. Herceptin is made by recombinant DNA technology in then injected into patient. It increases the survival of people with cancer by 20-25 months in late stages. However, cancers usually develop resistance to trastuzumab. Approx. 70% of HER2+ patients do not respond to treatment. In fact resistance is developed rapidly by treatment, in virtually all patients. The antibody treatment is also expensive (\$100,000 per year). Another monoclonal antibody, **Pertuzumab**, which inhibits dimerization of HER2 and HER3 receptors, was approved by the FDA in 2012.

Breast cancer vaccines mimic the success of Herceptin by immunizing with either large recombinant Her2 protein fragments or various antigenic peptides (single or mixture). The objective is to induce the production of antibodies in the patients. This will reduce the cost of producing and injecting Herceptin and also reduce Her2 resistance. **NeuVax**, developed by Galena Biopharma, is a peptide-based vaccine aimed at preventing or delaying the recurrence of breast cancer in cancer survivors who achieve remission after standard of care treatment (e.g., surgery, radiation, chemotherapy). It consists of the **E75 synthetic peptide (Her2 369-377)** initially isolated from HER2/neu proto-oncogene combined with the immune adjuvant, granulocyte macrophage colony stimulating factor (rhGM-CSF from yeast).

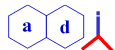
GP2 peptide (654-662) is a 9 aa HLA-A2-restricted peptide derived from the transmembrane domain of HER2. It is as effective as E75 at inducing a CTL response, suggesting that it might be more immunogenic than E75. A phase I clinical study using GP2 in combination with GM-CSF is ongoing. **AE37 peptide** (776-790 aa) is a HER2/Neu-derived epitope linked to li-Key peptide (li-Key/HER2/neu hybrid peptide or AE37). A Phase I clinical trial administering AE37, a HER2/neu Class II epitopes to disease-free, NN breast cancer patients showed that the li-Key moiety, a 4-amino acid (LRMK) epitope from the MHC class II-associated invariant chain (li protein), increases T-helper cell stimulation. **QIAKGMSYL** is a peptide, derived from the ECD of Her2. It is naturally presented by various HER2 positive cell lines.

Multi peptide vaccines: These peptides are derived from the ICD and ECD of Her2. ECD-derived peptides (p42 (aa 42–56), p98 (aa 98–114) and p328 (aa 328–345); ICD-derived peptides (p776 (aa 776–790), p927 (aa 927–941) and p1166 (aa 1166–1180); Derived from both domains: p369 (aa 369–386), p688 (aa 688–703) and p971 (aa971–984). Peptides derived from the **HER-2/trastuzumab interface** Peptides derived from the HER-2/trastuzumab interface (**563CYC**) is a cyclic peptide containing the sequences 563- 598); **585CYC** is a cyclic peptide containing sequences 585-598. **597CYC** is a cyclic peptide containing sequences 597-626. The last a.a cys is mutated to Leu so as to prevent interference with natural disulphide formation. **613** is a peptide containing sequences 613-626.

Her2 Protein Vaccines: HER2 ICD (aa 676– 1255): phase I clinical trial showed T cell response specific for HER2 ICD in 89% of immunized patients and 82% developed anti-HER2 IgGs. **dHER2** is a recombinant anti-HER2 protein-based vaccine, made of the HER2 ECD and a portion of ICD this vaccine was evaluated in 15 patients with breast cancer and showed that Abs specific for HER2 ECD and ICD developed after 4 immunizations. **CHP-HER2** (aa1–146) is a recombinant vaccine composed of a truncated HER2 protein encoding aa terminal) complexed to a delivery system consisting of Cholesteryl Pullulan nanogels (CHP). **MVF-HER-2 vaccine:** Phase 1: HER2/neu peptide vaccine comprising measles virus epitope MVF-HER-2 (266-296) and MVF-HER-2 (597-626) emulsified with nor-MDP in ISA 720.

Her2 DNA Vaccines: DNA vaccines encode a modified human HER2 protein without tyrosine kinase activity. All of them induced both cellular and humoral immune responses leading to in vivo tumor protection. **pE2A** which encodes a full length HER2 in which Lys753 has been substituted by Ala to remove the ATP-binding Lys residue; **pE2TM** encodes the HER2 signal peptide, extracellular and transmembrane domains but not the intracellular ; **psecE2** encodes the 1–505) of ECD as a secreted protein. **pcytE2** (i.e., HER2 without signal peptide) elicited only a CD8+ TL response; **p185**, encodes HER2 ECD and the TM domain, was effective in inhibiting carcinogenesis in a transgenic mouse model; **MVA-BN-HER2** formed by a non-replicating viral vector encoding a truncated form of HER2 protein (without its ICD) and two universal T epitopes of the tetanus toxin used to boost the immune system.

All of the above vaccines (her2 peptides, protein or DNA) must be able to induce robust antibodies to Her2 protein. It will also be important to identify subtype of her2-antibody as a result of vaccine. ADI has developed antibody ELISA kits for animals and humans to determine the efficacy of various existing Her2 vaccines and test new vaccines. ADI is further expanding the antibody ELISAs to measure IgG (and IgG1, IgG2a, IgG3, IgG4) and IgM classes. ELISA kits are also available to measure the her2 in animals and humans and if patients are producing antibodies to Her2 in response to Herceptin immunotherapy. We have also developed ELISA kits to detect if cancer patients or animals already have autoantibodies to her2 as a results tumor overexpressing her2.



Breast Cancer Vaccines: Antibody ELISA Kits, Recombinant Proteins, Peptides and Antibodies

Items Description	Species	Cat#	
Herceptin /Trasuzumab ELISA Kit for serum or biological buffers	Human/Mouse /Rat	200-510-HLG	
Human Anti-Herceptin /Trasuzumab Antibody (HAHA) ELISA Kit	Human	200-520-HAG	
Her2/neu /Erb2/CD340 protein ELISA kit, 96 tests	Human	200-530-HER	

Items Description	Species	IgG Specific Cat#	IgM Specific Cat#
Her2 Vaccine (Anti- Her2 Protein , EC-Domain) ELISA Kit	Human	200-600-HRH	200-610-HRM
	Mouse	200-620-HRH	200-630-HRM
Her2 Vaccine (E75 peptide) IgG ELISA kit	Human	200-640-HRH	200-650-HRM
	Mouse	200-660-HRH	200-670-HRM
Her2 Vaccine (AE37 peptide) IgG ELISA kit	Human	200-700-HRH	200-710-HRM
	Mouse	200-720-HRH	200-730-HRM

Breast Cancer Vaccines: Antibody Recombinant Proteins, and Peptides

Catalog#	Product Description	Product Type	Catalog#	Product Description	Product Type
HER21-R-10	Recombinant (HEK) human Her2/Erb2/Neu (1-652)-hlgG-Fc fusion protein	Protein	HER2-776-P	HER2 peptide, (776 – 790 fused with LRMK, C-Term), GP2 vaccine candidate	peptides
HER22-R-5	Recombinant (sf9) human Her2/Erb2/Neu (676-1255)-GST fusion protein	Protein	HER2-MP1	HER2 multi peptide, (369-386, 688-703,971-984); vaccine candidate	peptides
HER23-R-10	Recombinant (HEK) human Her2/Erb2/Neu (1-652)-his tag	Protein	HER2-MP2	HER2 multi peptide, (776-790,927-941,116-1180); vaccine candidate	peptides
HER24-R-10	Recombinant (HEK) mouse Her2/Erb2/Neu (1-653)-his tag protein	Protein	HER2-MP3	HER2 multi peptide, (42-56,98-114,328-345); vaccine candidate	peptides
HER25-R-10	Recombinant (HEK) mouse Her2/Erb2/Neu (1-653)-hlgG1-Fc fusion	Protein	SM-101000-5	EGFR/HER2 kinase inhibitor (>99%, M.wt 485.94) (Afatinib/BIBW-2992	Chemical
HER26-R-10	Recombinant (HEK) rat Her2/Erb2/Neu (4-656)-his tag fusion protein	Protein	SM-101010-5	Inhibitor of EGFR/HER family (Her1, Her2, Her3 or Pan Her-inhibitor) (BMS-59926/AC480, Mol wt 567.01, >99%)	Chemical
HER27-R-10	Recombinant (HEK) rat Her2/Erb2/Neu (4-656)-his tag fusion protein	Protein	SM-101020-10	Inhibitor of EGFR/HDAC/Her2 (CUDC-101 Mol wt 434.49, >99%)	Chemical
HER28-R-10	Recombinant (HEK) rat Her2/Erb2/Neu (4-656)-hlgG1-Fc fusion protein	Protein	SM-101040-5	Cell permeable Inhibitor of EGFR/ERB family/Her2 (Neratinib/HKI-272,	Chemical
HER29-R-10	Recombinant (HEK) monkey/rhesus Her2/Erb2/Neu (1-652)-his tag protein	Protein	SM-101050-100	Cell permeable Inhibitor of EGFR2/FGFR/PDGFR/JAK1/Her2	Chemical
HER30-R-10	Recombinant (HEK) monkey/rhesus Her2/Erb2/Neu (1-652)-hlgG1-Fc protein	Protein	SP-102029-5	Herpes Virus Inhibitor 1 (AA: Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu) (MW: 920.46)	Pure Peptide
HER31-M	Rabbit mono anti-human Her2/Erb2/Neu (1-652) protein IgG	Antibodies	SP-51177-1	HER2/neu (869-877) peptide	Pure Peptide
HER32-A	Rabbit Anti-human Her2/Erb2/Neu (1-652) protein IgG	Antibodies	SP-52260-1	HER2/neu(654-662) GP2	Pure Peptide
HER33-M	Mouse mono anti-monkey/rhesus Her2/Erb2/Neu (1-652) protein IgG	Antibodies	SM-101060-25	Lapatinib Ditosylate (GW572016, GW2016, Tykerb, Tyverb), Autophos. Inhibitor of Her2/Erb2 (mol wt 925; >98%)	Chemical
HER34-A	Rabbit Anti-monkey/rhesus Her2/Erb2/Neu (1-652) protein IgG	Antibodies	SM-101070-10	Canertinib (CI-1033), kinase Inhibitor of Her2/Erb2/EGFR (mol wt 485; >98%)	Chemical
HER2-369-P	HER2 peptide, (369 – 377), E 75 vaccine	peptides	SM-101080-5	CP-724,714, Potent and selective Inhibitor of Her2/Erb2 (mol wt 469; >98%)	Chemical
HER2-563-P	HER2 peptide, cyclic, (563-598, cys- cys disulphide bond); vaccine candidate	peptides	SM-101090-5	AZD8931, reversible and competitive Inhibitor of Her2/Erb2/EGFR	Chemical
HER2-585-P	HER2 peptide, cyclic, (585-598, cys- cys disulphide bond); vaccine candidate	peptides	SM-101100-5	AEE788 (NVP-AEE788), dual Inhibitor of Her2/Erb2/EGFR (mol wt 440; >98%)	Chemical
HER2-597-P	HER2 peptide, cyclic, (597-626, cys- cys disulphide bond) vaccine candidate	peptides	SM-101110-10	Mubritinib (TAK-165), potent Inhibitor of Her2/Erb2 (IC50=6 nm	Chemical
HER25-R-10	HER2/ErB2 recombinant protein (1-652, extracellular domain), Recombinant	peptides	SM-101120-5	Arry-380, Oral, potent Inhibitor of Her2/Erb2 Tyr kinase (IC50=8 nM; mol wt 869; >98%)	Chemical
HER2-613-P	HER2 peptide, cyclic, (613-626, cys- cys disulphide bond); vaccine candidate	peptides	SM-101130-5	Tak-285, dual Inhibitor of Her2/EGFR Tyr kinase (IC50=17 nM; mol wt 547; >98%)	Chemical
HER2-654-P	HER2 peptide, (654 – 662), GP2 vaccine	peptides	SM-101140-25	Lapatinib, Inhibitor of Her2/EGFR (IC50=10 nM; mol wt 581; >98%)	Chemical
HER26-R-10	HER2/ErB2 recombinant protein (676– 1255, intracellular domain), Recombinant	Protein			

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