# Trends in Circulating Tumor Cells in Multiple Adjuvant Trials of HER2-Specific Peptide Vaccines in Breast Cancer Patients

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## Background

Circulating Tumor Cells (CTCs) are an independent prognostic factor of overall survival in metastatic breast cancer and data suggests a role for CTCs predicting recurrence in patients with non-metastatic breast cancer.

We are conducting phase II trials evaluating 3 HER2-specific vaccines (E75 (NeuVax), AE37, GP2) in the adjuvant setting and have previously published "proof of principle" data suggesting a potential role for CTCs as a marker of response to adjuvant immunotherapy.

In previous studies, we have shown CTCs decrease after primary series and booster peptide vaccinations.

This study was undertaken to evaluate updated data on CTCs in these trials.

## Methods

Node positive or high-risk node negative, disease-free breast cancer patients with any level of HER2 expression were enrolled after standard treatments.

In the AE37 and GP2 trials, patients were randomized to either peptide + GM-CSF(the vaccine group, VG) or GM-CSF alone (the control group, CG). In the NeuVax (nelipepimut-S) trial, HLA-A2/A3+ pts were assigned to the vaccine group (VG) and HLA-A2/A3- pts were followed prospectively as a control group (CG). VG patients in all trials received six, monthly intradermal inoculations in the primary vaccine series followed by booster inoculations every 6 months (B1-B7).

CTCs were enumerated from blood samples using the CellSearch System (Veridex, LLC Warren, NJ). After establishing baseline CTCs, those with ≥1 CTC had subsequent measurements taken at R3, R6 and with each booster. Patients with multiple data points were divided into those with increased/stable (I/S) or decreased (D) CTCs counts. Immunologic response of each group (I/S v D) in the NeuVax trial was measured *in vivo* with delayed-type hypersensitivity (DTH) and *in vitro* with dimer assays.

## Results

Combining all trials, CTCs were measured in 96 patients (74VG, 22CG) with 56 (57%) having ≥1 CTC at baseline. 44 (39VG, 5CG) of 56 patients had more than one CTC data point.

VG patients were more likely to have a decrease (24/39) in CTCs than were CG patients (59% v 20%, p=0.16).

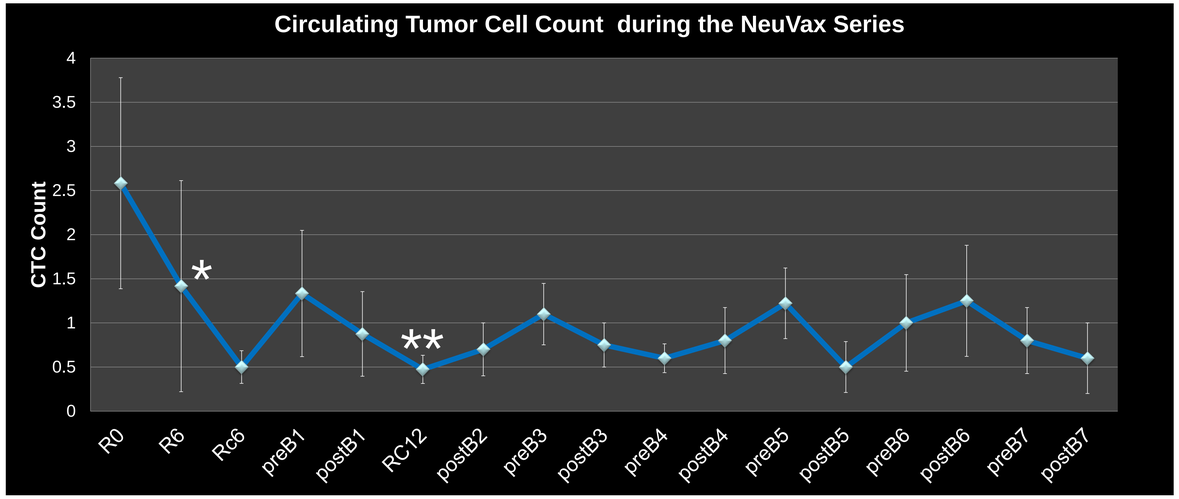
Analyzing NeuVax vaccinated patients, 26 had more than one data point. 16 of 26 patients had decreased CTCs with an average decrease of 3.06±0.93(SEM). The average number of CTCs decreased from R0 to R6 and all post primary vaccine series time points (12/16 to zero). **See Figure 1.**

Compared to those patients with increased (n=6) or stable (n=4) CTCs, patients with decreasing CTC counts (n=16) demonstrated increased post primary vaccine series DTH and dimer responses. **See Figure 2 and 3.**

## Conclusions

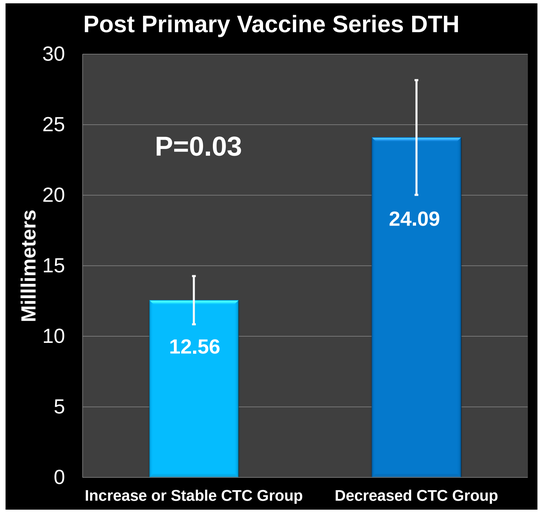
**Adjuvant Breast Cancer vaccines decrease the number of CTCs, and our data suggests a correlation between decreasing CTCs and enhanced standard immunologic response assays. Monitoring CTC trends may be clinically useful in the adjuvant setting as a surrogate for response to peptide vaccines. Importantly, in some patients, CTCs persist suggesting that breast cancer is a chronic disease.**

## Figure 1

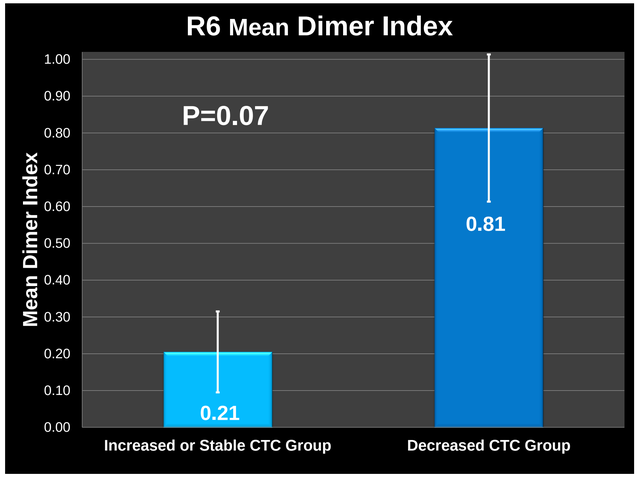



*** Decrease in CTCs from R0 to RC6 (2.58 v 0.39, p<0.05) **Decrease in CTCs from R0 to RC12 (2.58 v 0.47, p<0.04)**

## Figure 2




## Figure 3




## Disclosures

Dr. Peoples has partial inventor rights to E75. AE37, and GP2. Several patents have been licensed for commercial development. He is entitled to financial proceeds associated with these licenses per Federal policy. Dr. Peoples also consults in the development of the vaccines. All other authors have no relevant financial disclosures.

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