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Status of *HER-2* gene amplification in breast cancers from Native American women

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Letter to the editor

In the United States, one in eight women will be diagnosed with breast cancer during her lifetime. The American Cancer Society estimates that in 2008, approximately 182,460 new female breast cancer cases will be diagnosed and 40,480 women will die from this disease [1]. However, breast cancer incidence and mortality rates in females vary across ethnic and racial groups. The National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program indicates that incidence rates for breast cancer among Native American women are significantly lower than the Caucasian population [2]. Yet, Native American women tend to present to their physician with larger, more advanced-stage tumors that lead to lower survival rates, when compared to their Caucasian counterparts [3][sn1]. Moreover, mortality rates for Native American women are not decreasing as they are for the Caucasian population [1,4]. It has been particularly difficult to study the molecular basis of breast cancer in the Native American people because the population is small and has limited access to healthcare. Therefore, this report describes a small cohort study of 60 breast cancer samples from Native American patients and determines the status of *HER-2* amplification.

Amplification of *HER-2*, a proto-oncogene, has been observed in many types of cancers, occurring most frequently in breast cancer [5]. The amplification of *HER-2* is exhibited in 25–30% of the breast cancers in the general population [6,7]. Breast cancers that have *HER-2* amplification are associated with a more aggressive phenotype, including larger tumor size, higher-grade, increased mitotic activity, lower levels of the hormone receptors, and a poorer prognosis and shorter overall survival rate for the patient [7].

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Since Native American women with breast cancer tend to have a less favorable prognosis and a poorer five-year survival rate than that of the general population [4,8], we hypothesized that the more aggressive breast cancer phenotype observed in Native American women might be due to a higher frequency of *HER-2* gene amplification in their breast cancers. Therefore, the goal of the work presented here is to estimate *HER-2* gene amplification prevalence in breast cancers from Native American women and to compare this rate to the general population. In addition, associations between *HER-2* gene amplification and other tumor characteristics, such as the status of the estrogen receptor (ER) and progesterone receptor (PR), tumor type, grade, number of nodes involved, greatest tumor dimension, and patient age, were evaluated.

A total of 60 [p2] formalin-fixed, paraffin-embedded breast cancer specimens from Native American patients were obtained from the tissue archives of Sanford Health, Sioux Falls, SD and the Rapid City Regional Hospital, Rapid City, SD. The patients were identified as Native American using the information provided by the patients in the medical registry. All breast cancer cases studied were diagnosed between the years 2000 and 2006. These tumor specimens were de-identified, and an approved IRB protocol was followed. The results from this work were presented at the Native American Health Summit in Sioux Falls, SD in 2007. A cross-sectional study was done to screen for the presence of *HER-2* gene amplification by fluorescence *in situ* hybridization (FISH) analysis using the Paraffin Pretreatment Kit II (Abbott Molecular-Vysis Incorporated, Downers Grove, IL) and the United States FDA approved PathVysion™ *HER-2* DNA Probe Kit (Abbott Molecular-Vysis Incorporated). The kit used two DNA probes labeled with fluorophores, which allowed for the simultaneous detection of *HER-2* and chromosome 17 gene copy numbers. The locus specific identifier (LSI®) *HER-2* DNA probe, labeled in SpectrumOrange™, spans the full-length of the *HER-2* gene and is a 190 kilobase (kb) sequence specific for the *HER-2* gene locus (17q11.2–q12). The centromeric enumeration probe 17 (CEP® 17) is labeled in SpectrumGreen™ and is a 4.5 kb sequence directed against the alpha-satellite DNA of the centromeric region of chromosome 17 (17p11.1–q11.1). The CEP 17 probe served as an internal control, allowing for the relative copy number of *HER-2* to be determined to help distinguish between gene duplication and gene amplification (Abbott Molecular-Vysis Incorporated).

Scoring of *HER-2* and CEP 17 signals was restricted to cancer cells due to the prior identification of these cells on the corresponding H&E stained slide by a pathologist. *HER-2* and CEP 17 signals were counted in at least 20 interphase nuclei and were reported as a ratio of average *HER-2* copy numbers to average copy numbers of CEP 17. The slides were scored at 750x magnification, and cases with an average ratio ≥ 2 were considered amplified. If the ratio was near the cut-off value of 1.8–2.2, an additional 20 nuclei were evaluated and the ratio was recalculated. Two clinically trained laboratory personnel (LDR & SEH; PathVysion® *HER-2* Training Program, Abbott Molecular-Vysis, Incorporated) independently counted each slide, with the results reported as an average of these two independent scores[p3].

Sixty breast cancer cases from the Native American population, which represents all currently available material, were screened for *HER-2* gene amplification using FISH analysis. Fig. 1 displays representative images of an unamplified and an amplified breast cancer case on which FISH was performed. The pathology and clinical data for each patient used in this study are summarized in Table 1. Of the 60 cases screened, 13 were classified as having *HER-2* gene amplification, and 47 were unamplified. Thus, 21.7% of the studied breast cancers from the Native American population exhibited *HER-2* gene amplification with a 95% confidence interval from 11.2% to 32.1%. This amplification proportion is not significantly different from the general population reported in previous studies: 53 out of 189 (28.0%) by Slamon, et al. ($p = 0.33$ in z-test), 657 out of 2502 (26.3%) by Press, et al. ($p = 0.42$ in z-test) [6,7]. Therefore, we did not detect an increase of *HER-2* amplification in breast cancers from Native American women compared to the general population. If these results were used prospectively to design

another study to examine *HER-2* gene amplification in the Native American population, there would be 80% power to detect prevalence below 8% or above 38% based on 60 samples and a two-sided critical level of 0.05 using an exact binomial distribution.

In addition, associations between *HER-2* gene amplification and tumor characteristics for the 60 breast cancer samples were analyzed using two-way contingency tables with Fisher's exact test. Statistical analyses revealed that *HER-2* gene amplification in breast cancers from Native American women is associated with the absence of the ER and PR, which are similar findings to those reported for the general population [7]. Samples with ER or PR present were 87% or 84%, respectively, less likely to have *HER-2* amplification than samples without ER or PR. The greatest tumor dimension, number of nodes involved, tumor type, grade of tumor, and age were not significant factors in the analyses.

It is well known that incidence rates and biological characteristics of breast cancer vary largely among ethnic groups [2]. For better diagnosis and treatment options, it is necessary to examine tumor characteristics specific to each ethnic group and associate this information with the occurrence of high-grade tumors. However, biological risk factors correlated with specific ethnic groups have been insufficiently elucidated, largely because it is difficult to separate a person's biological background from other societal risk factors such as poverty, availability of health insurance, and lack of access to screening facilities. Although our study revealed a difference of 6.3% [p6] between the two populations, with breast cancer samples from Native American women having a lower rate of *HER-2* amplification, our statistical analysis indicated that this is not a significant difference between the two populations based on the available sample size. We provided evidence here for an indistinguishable rate of *HER-2* gene amplification in breast cancers between Native American women and the general population, suggesting that *HER-2* amplification does not play a role in the observed breast cancer disparity between Native American women and the general population. Therefore, there should be other factors that result in the more aggressive phenotype observed in Native American women, such as socioeconomic factors, insurance coverage, access to healthcare, reduced prevalence of cancer screening, *BRCA1/BRCA2* mutations or the status of ER and PR.

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Fig. 1.

Representative images of breast tumor specimens from Native American women that were screened for the presence of *HER-2* amplification. The blue color indicates a DAPI nuclear counterstain. The green signals are the hybridized chromosome 17 control probes, while the orange signals are the hybridized *HER-2* probes. Images A and B were acquired at 750X. A. Native American breast tumor with normal numbers of the *HER-2* gene. This figure illustrates a breast tumor specimen with normal *HER-2* levels by the presence of a 1:1 ratio of *HER-2* signals (orange) to chromosome 17 control signals (green). B. Native American breast tumor with amplification of the *HER-2* gene indicated by the presence of a *HER-2* to chromosome 17 ratio greater than 2:1 (orange:green). C. Magnification of the area in the yellow box in panel A. D. Magnified image of the area in the yellow box from panel B.

Table 1
[p7]. Breast Tumors Clinicopathological Characteristics (N=60)

Feature	n (%)
Age	
<30 years	1 (1.7)
30–39 years	4 (6.7)
40–49 years	14 (23)
50–59 years	13 (22)
60–69 years	15 (25)
70–79 years	8 (13)
≥80 years	3 (5.0)
Unknown	2 (3.3)
Tumor Type¹	
<i>In situ</i>	6 (10)
Invasive	21 (35)
Metastatic	23 (38)
Unknown	10 (17)
Tumor Size	
1–10 mm	12 (20)
11–20 mm	17 (28)
21–50 mm	19 (32)
> 50 mm	6 (10)
Unknown	6 (10)
Axillary Nodes Positive for Carcinoma	
0 nodes	24 (40)
1–3 nodes	15 (25)
4–9 nodes	5 (8.3)
≥10 nodes	5 (8.3)
Unknown	11 (18)
Grade	
I (well differentiated)	7 (12)
II (moderately differentiated)	17 (28)
III (poorly differentiated)	36 (60)
Estrogen Receptor Status	
Positive	39 (65)
Negative	19 (32)
Unknown	2 (3.3)
Progesterone Receptor Status	
Positive	37 (62)
Negative	21 (35)
Unknown	2 (3.3)

¹ Tumors were classified into three types: *in situ*, invasive, and metastatic. Metastatic cancers were defined as having one or more lymph nodes positive for cancer.