NELL1 is differentially expressed in the tumors of breast cancer patients treated with trastuzumab.

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Trastuzumab (Herceptin) is a monoclonal antibody targeting the extracellular domain of the human epidermal growth factor receptor 2 (HER2)¹ utilized for the treatment of adjuvant and metastatic breast cancer² in the United States and worldwide. We mined published microarray data^{3,4} to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified the gene encoding neural epidermal growth factor (EGF)-like 1, NELL1, among the genes most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab. The primary tumors of breast cancer patients treated with trastuzumab expressed higher levels of NELL1 messenger RNA than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of NELL1 in primary tumors of the breast, demonstrating increased expression of a growth factor-like molecule whose increased primary tumor expression is significantly correlated with worse overall survival in patients with rhabdomyosarcoma⁵ as a direct transcriptional consequence of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, NELL1, neural EGF-like 1, systems biology of breast cancer, targeted therapeutics in breast cancer.

The mechanism of action ascribed to trastuzumab function is thought to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2 (HER2)¹, but the transcriptional consequences resulting from signaling downstream of trastuzumab binding to HER2 is less well understood. Through unbiased, systems-level comparative transcriptome analysis of published microarray data^{3,4}, we report here that NELL1 is a differentially expressed gene in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of NELL1 than did those of patients not treated with trastuzumab.

Methods

We used datasets GSE130788³, and GSE114082⁴ for this differential gene expression analysis of the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE130788 was generated using Agilent-014850 Whole Human Genome Microarray 4x44K G4112F technology with *n*=11 primary breast tumors from patients treated with trastuzumab and *n*=36 primary tumors from patients treated with trastuzumab, analysis performed using platform GPL6480. GSE114082 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2 expression beadchip technology with *n*=17 primary breast tumors from patients before trastuzumab treatment and *n*=17 primary tumors from patients after trastuzumab treatment, analysis performed using platform GPL14951. The Benjamini and Hochberg method of *p*-value adjustment was used for ranking of differential expression but raw *p*-values were used to assess statistical significance of global or multiplexed differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used. A statistical test was performed to evaluate whether NELL1 gene expression was significantly between primary breast tumors of patients receiving trastuzumab using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455).

Results

We performed unbiased comparative transcriptome analysis using published microarray data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of patients treated with trastuzumab (Herceptin).

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We identified the neural epidermal growth factor (EGF)-like 1, encoded by NELL1, as among the genes whose expression changed most significantly transcriptome-wide in the tumors of breast cancer patients treated with trastuzumab in a microarray dataset³ (Table 1). When sorting each of the genes expressed in the primary tumors of breast cancer patients treated with trastuzumab based on significance of change as compared to that of patients not treated with trastuzumab, NELL1 ranked 16 out of 41000 total transcripts (Table 1), equating to 99.96% differential expression. NELL1 differential expression in the tumors of patients treated with trastuzumab trended towards statistical significance (Table 1; *p*=3.97E-81).

We turned to a second microarray dataset to validate our findings of NELL1 differential expression in the patients treated with trastuzumab. The tumors compared in this dataset were sampled from the same patient before and after a single, brief exposure to trastuzumab, not comparing tumors from different patients treated with a course of trastuzumab or not. Thus, while differential expression of genes identified based on comparison of tumors from different patients based on history of trastuzumab treatment can result from a variety of sources, including gene expression changes resulting from tumor evolution over the course of treatment, changes identified in this dataset, occurring after only a single

1 treatment of trastuzumab and by comparing tumors from the same patients before and after administration
2 of trastuzumab, are more likely to be a direct transcriptional result of trastuzumab binding events. We
3 found again that NELL1 was among the genes most differentially expressed in the tumors of patients
4 treated with trastuzumab, here, in patients administered a single dose of trastuzumab (Table 2). When
5 sorting each of the genes expressed in the tumors of patients administered a single dose of trastuzumab,
6 NELL1 ranked 3940 out of 29377 total transcripts (Table 2), equating to 86.6% differential expression.
7 Differential expression of NELL1 in the primary tumors of breast cancer patients following treatment
8 with trastuzumab was statistically significant (Table 2; $p=3.13E-03$).

9 **NELL1 is expressed at significantly higher levels in the tumors of breast cancer patients treated**
10 **with trastuzumab than in the tumors of those not treated with trastuzumab.**

11 We obtained exact mRNA expression levels for NELL1 from tumors of patients treated with
12 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate direction
13 and statistical significance of change in expression of NELL1 based on history of trastuzumab treatment.
14 NELL1 was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to
15 those of patients not treated with trastuzumab, and this difference was deemed statistically significant
16 (Figure 1; $p<0.0001$).

17 Thus, we found using published microarray data^{3,4} that NELL1 was among the genes most
18 differentially expressed in the tumors of patients treated with trastuzumab, that NELL1 was expressed at
19 higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with
20 trastuzumab, and that this transcriptional change was likely a direct result of trastuzumab treatment as
21 opposed to a gene expression change associated with clonal selection and evolution of the tumor across
22 the course of treatment.

23 **Discussion**

24 We provided evidence through analysis of published microarray data that neural EGF-like 1,
25 NELL1, is a differentially expressed gene in the tumors of patients treated with trastuzumab, and
26 expressed at significantly higher levels in the primary tumors of breast cancer patients treated with
27 trastuzumab as compared to patients not treated with trastuzumab. Thus, it appears that trastuzumab
28 administration in patients with breast cancer results in increased primary tumor expression of growth
factor-like molecule⁶ whose increased expression is significantly correlated with decreased overall
survival in patients with rhabdomyosarcoma⁵.

References

1. Hudis, C.A., 2007. Trastuzumab—mechanism of action and use in clinical practice. *New England Journal of Medicine*, 357(1), pp.39-51.
2. Herceptin (Trastuzumab). Indications. <https://www.herceptin.com/hcp/indications.html>.
3. GSE130788. A Phase II Randomized Trial of Neoadjuvant Trastuzumab or Lapatinib or the Combination of Trastuzumab and Lapatinib, Followed by Docetaxel and Carboplatin with Trastuzumab and/or Lapatinib in Patients with HER2+ Breast Cancer. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE130788>. Hsiaowang Chen. UCLA, Department of Medicine. Santa Monica, CA.
4. Triulzi, T., Regondi, V., De Cecco, L., Cappelletti, M.R., Di Modica, M., Paolini, B., Lollini, P.L., Di Cosimo, S., Sfondrini, L., Generali, D. and Tagliabue, E., 2018. Early immune modulation by single-agent trastuzumab as a marker of trastuzumab benefit. *British journal of cancer*, 119(12), pp. 1487-1494.
5. Tombolan, L., Poli, E., Martini, P., Zin, A., Romualdi, C., Bisogno, G. and Lanfranchi, G., 2017. NELL1, whose high expression correlates with negative outcomes, has different methylation patterns in alveolar and embryonal rhabdomyosarcoma. *Oncotarget*, 8(20), p.33086.
6. Aghaloo, T., Cowan, C.M., Chou, Y.F., Zhang, X., Lee, H., Miao, S., Hong, N., Kuroda, S.I., Wu, B., Ting, K. and Soo, C., 2006. Nell-1-induced bone regeneration in calvarial defects. *The American journal of pathology*, 169(3), pp.903-915.

Rank	ID	p-value	t	B	Gene	Gene name
16	A_23_P251151	3.97E-81	32.282792	174.399784	NELL1	neural EGFL like 1

Table 1: NELL1 is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

The rank of differential expression, probe ID, p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene
3940	ILMN_1745817	3.13E-03	3.1681441	-1.92162	NELL1

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of NELL1 in the primary tumors of patients with breast cancer.

The rank of differential expression, probe ID, p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Figure 1: NELL1 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab.

The mRNA expression level of NELL1 is graphically depicted in this chart in the primary tumors of breast cancer patients not treated with trastuzumab (left) and in the primary tumors of breast cancer patients treated with trastuzumab (right), with mean mRNA expression level marked and the result of a statistical test evaluating significance of difference between NELL1 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.