Interleukin-1 α and the interleukin-1 receptor antagonist (IL-1RN) are differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

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Trastuzumab, a monoclonal antibody targeted against the human epidermal growth factor receptor 2 (HER2) is utilized for the treatment of human breast cancer^{1,2}, but a complete understanding of how tumor signal transduction is modulated by trastuzumab treatment is lacking. By mining published and public microarray and gene expression data^{3,4} from the primary tumors of patients treated with trastuzumab, we found that two components of the interleukin-1 cytokine signaling pathway, interleukin-1 α (IL-1 α) and the interleukin-1 receptor antagonist, IL-1RN, were among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab. While IL-1 α cytokine levels were increased, IL-1RN levels were decreased. Thus, the administration of trastuzumab in patients with breast cancer is associated with increased expression of a cytokine demonstrated to promote breast cancer progression and decreased expression of an interleukin receptor antagonist with metastasis suppressive properties in breast cancer human xenograft models *in vivo*^{5,6}.

Keywords: breast cancer, trastuzumab, IL-1 α , IL-1RN, IL1RN, IL1RA, IL1RN, systems biology of breast cancer, targeted therapeutics in breast cancer.

The mechanism of action by which trastuzumab exerts its therapeutic function in human breast cancer is said to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor 2 (HER2)², possibly resulting in decreased cellular proliferation⁷. Transcriptional modulation resulting from trastuzumab binding events in the primary tumors of treated patients is less well understood. We mined published and public microarray and gene expression data^{3,4} generated using primary tissues from patients treated with trastuzumab to compare tumor global gene expression with that of patients not treated with trastuzumab. Using this unbiased, systems level methodology, we identified differential expression of IL-1 α and IL-1RN in primary tumors of the breast in patients treated with trastuzumab.

Methods

We used datasets GSE38057³ and GSE130788⁴ for this differential gene expression analysis of the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was generated using DASL Human Cancer Panel by Probe technology with $n=11$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=36$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in addition to trastuzumab; analysis was performed using platform GPL5826. 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. 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 IL-1 α and IL-1RN 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 comparative transcriptome analysis of primary tumors from patients treated or not with trastuzumab (Herceptin), using published multiplexed gene expression data³ and a microarray dataset⁴, in order to identify the most significant transcriptional changes in the primary tumors of breast cancer patients following treatment with trastuzumab.

IL1RA is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that the IL-1 α , encoded by IL1RA, was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab³. When sorting each of the genes expressed in the primary tumors of breast cancer patients treated with trastuzumab based on significance of difference in expression as compared to the primary tumors of breast cancer patients not treated with trastuzumab, IL1RA ranked 5 out of 1506 total transcripts, equating to 99.7% statistical significance (Table 1). Differential expression of IL1RA in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.002338$).

We queried a second dataset⁶ to validate our findings of IL1RA differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with

trastuzumab, we again found that IL1RA was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of tumors from patients treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the genes expressed in the tumors of patients treated with trastuzumab based on significance of change as compared to the tumors of patients not treated with trastuzumab, IL1RA ranked 21324 out of 41000 total transcripts, equating to 48.0% differential expression. Though differential expression of IL1RA was less significant than greater than half of the transcriptome, differential expression of IL1RA in the primary tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=5.73E-03$).

IL1RA is expressed at higher levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for IL1RA from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of IL1RA change in the tumors of patients treated with trastuzumab. IL1RA expression was higher in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab (Figure 1). Enhanced expression of IL1RA in the primary tumors of trastuzumab-treated patients was statistically significant (Figure 1: $p=0.0035$). We calculated a mean fold change of 1.38 ± 0.41 when comparing the expression of IL1RA in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab.

IL1RN is differentially expressed in the primary tumors of patients treated with trastuzumab.

We also found that the interleukin-1 receptor antagonist, encoded by IL1RN, was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab³. When sorting each of the genes expressed in the primary tumors of breast cancer patients treated with trastuzumab based on significance of difference in expression as compared to the primary tumors of breast cancer patients not treated with trastuzumab, IL1RN ranked 8 out of 1506 total transcripts, equating to 99.5% statistical significance (Table 3). Differential expression of IL1RN in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 3; $p=0.004399$).

We queried a second dataset⁶ to validate our findings of IL1RN differential expression upon trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with trastuzumab, we again found that IL1RN was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of tumors from patients treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the genes expressed in the tumors of patients treated with trastuzumab based on significance of change as compared to the tumors of patients not treated with trastuzumab, IL1RN ranked 10629 out of 41000 total transcripts, equating to 74.1% differential expression. Though differential expression of IL1RN was less significant than greater than half of the transcriptome, differential expression of IL1RN in the primary tumors of patients treated with trastuzumab was statistically significant (Table 4; $p=5.48E-07$).

IL1RN is expressed at lower levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for IL1RN from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of IL1RN change in the tumors of patients treated with trastuzumab. IL1RN expression was lower in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated

1 with trastuzumab (Figure 2). Decreased expression of IL1RN was statistically significant (Figure 2:
2 $p=0.0392$). We calculated a mean fold change of 0.61 ± 0.28 when comparing the expression of IL1RN
3 in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with
4 trastuzumab.

5 Thus, we found that the IL-1 cytokine signaling components IL-1 α and IL1-RN, encoded by
6 IL1RA and IL1RN, were among the genes most differentially expressed in the primary tumors of patients
7 with breast cancer. While expression of IL-1 α was higher in the primary tumors of patients treated with
8 trastuzumab as compared to those not treated, IL-1RN expression was lower.

9 **Discussion**

10 We provided evidence here that IL-1 α and IL-1RN, encoded by IL1RA and IL1RN were among
11 the most differentially expressed genes transcriptome-wide in the primary tumors of patients treated with
12 trastuzumab. While we observed significantly enhanced expression of IL-1 α in the primary tumors of
13 patients treated with trastuzumab, we observed significantly reduced expression of IL-1RN in the tumors
14 of patients treated with trastuzumab. Thus, administration of trastuzumab in patients with breast cancer
15 results in increased expression of IL-1 family signaling component that promotes breast cancer
16 progression⁵, but decreased expression of an IL-1 family signaling component that restricts metastasis *in*
17 *vivo*⁶.
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References

1. Slamon, D., Eiermann, W., Robert, N., Pienkowski, T., Martin, M., Press, M., Mackey, J., Glaspy, J., Chan, A., Pawlicki, M. and Pinter, T., 2011. Adjuvant trastuzumab in HER2-positive breast cancer. *New England Journal of Medicine*, 365(14), pp.1273-1283.
2. Hudis, C.A., 2007. Trastuzumab—mechanism of action and use in clinical practice. *New England Journal of Medicine*, 357(1), pp.39-51.
3. Duchnowska, R., Jassem, J., Goswami, C.P., Dundar, M., Gökmen-Polar, Y., Li, L., Woditschka, S., Biernat, W., Sosińska-Mielcarek, K., Czartoryska-Arłukowicz, B. and Radecka, B., 2015. Predicting early brain metastases based on clinicopathological factors and gene expression analysis in advanced HER2-positive breast cancer patients. *Journal of Neuro-oncology*, 122(1), pp.205-216.
4. 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](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE130788). Hsiaowang Chen. UCLA, Department of Medicine. Santa Monica, CA.
5. Kuan, E.L. and Ziegler, S.F., 2018. A tumor–myeloid cell axis, mediated via the cytokines IL-1 α and TSLP, promotes the progression of breast cancer. *Nature immunology*, 19(4), pp.366-374.
6. Elaraj, D.M., Weinreich, D.M., Varghese, S., Puhlmann, M., Hewitt, S.M., Carroll, N.M., Feldman, E.D., Turner, E.M. and Alexander, H.R., 2006. The role of interleukin 1 in growth and metastasis of human cancer xenografts. *Clinical Cancer Research*, 12(4), pp.1088-1096.

Rank	ID	p-value	t	B	FC	Gene
5	GI_27894329-S-1	0.002338	-3.21231	-2.65	1.38 ± 0.41	IL1A

Table 1: IL1RA 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, fold change of expression in IL1RA between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
21324	A_23_P72096	5.73E-03	-2.792912	-3.777184	IL1A	interleukin 1 alpha

Table 2: IL1RA 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, fold change of expression in IL1RA between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene
8	GI_27894318-A-3	0.004399	-2.98749	-2.92	0.61 ± 0.28	IL1RN

Table 3: IL1RN 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, fold change of expression in IL1RN between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
10629	A_32_P38125	5.48E-07	-5.177223	4.992525	IL1RN	interleukin 1 receptor antagonist

Table 4: IL1RN 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, fold change of expression in IL1RN between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Figure 1: IL1RA is expressed at higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of IL1RA 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 IL1RA expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.

Figure 2: IL1RN is expressed at lower levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of IL1RN 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 IL1RN expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.