Trastuzumab administration in patients with breast cancer is associated with increased primary tumor expression of IL-1 β .

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A complete understanding of how tumor signal transduction in human breast cancer is modulated by trastuzumab, a monoclonal antibody targeted against the human epidermal growth factor receptor 2 (HER2)^{1,2}, 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 the gene encoding interleukin-1 β ⁵, IL1 β , was among those most differentially expressed in the primary tumors of patients treated with trastuzumab. IL1 β was expressed at significantly higher levels in the tumors of patients treated with trastuzumab, indicating that a cytokine associated with metastasis to the bones in cancer models⁶⁻⁹ and correlated with relapse of disease to the bones in human breast cancer⁸ is produced at significantly higher quantities in the tumors of breast cancer patients treated with trastuzumab.

Keywords: breast cancer, trastuzumab, IL-1 β , systems biology of breast cancer, targeted therapeutics in breast cancer.

1 The mechanism of action by which trastuzumab exerts its therapeutic function in human breast
2 cancer is said to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human
3 epidermal growth factor receptor 2 (HER2)², possibly resulting in decreased cellular proliferation¹⁰.
4 Transcriptional modulation resulting from trastuzumab binding events in the primary tumors of treated
5 patients is less well understood. We mined published and public microarray and gene expression data^{3,4}
6 generated using primary tissues from patients treated with trastuzumab to compare tumor global gene
7 expression with that of patients not treated with trastuzumab. Using this unbiased, systems level
8 methodology, we identified differential expression and up-regulation of IL-1 β in primary tumors of the
9 breast in patients treated with trastuzumab.

10 **Methods**

11 We used datasets GSE38057³ and GSE66399⁴ for this differential gene expression analysis of the
12 transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was
13 generated using DASL Human Cancer Panel by Probe technology with $n=6$ primary breast tumors from
14 patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=35$
15 primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in
16 addition to trastuzumab, both of which developed brain metastases; analysis was performed using
17 platform GPL5826. GSE66399 was generated using Affymetrix Human Genome U133 Plus 2.0 Array
18 technology with $n=31$ primary breast tumors from patients treated with trastuzumab and $n=23$ primary
19 tumors from patients treated with trastuzumab, analysis performed using platform GPL570. The
20 Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression
21 but raw p -values were used to assess statistical significance of global or multiplexed differential
22 expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform
23 annotation was used. A statistical test was performed to evaluate whether IL-1 β gene expression was
24 significantly between primary breast tumors of patients receiving trastuzumab using a two-tailed,
25 unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene
26 expression in human breast cancer (Version 8.4.0)(455).

27 **Results**

28 **IL-1 β is differentially expressed in the primary tumors of patients treated with trastuzumab.**

1 We found that IL-1 β was among the genes whose expression was most significantly different in
2 the primary tumors of patients with breast cancer treated with trastuzumab using a multiplexed gene
3 expression dataset³. When sorting each of the genes expressed in trastuzumab-treated breast tumors
4 based on significance of difference in expression as compared to the primary breast tumors of patients not
5 treated with trastuzumab, IL-1 β ranked 12 out of 1506 total transcripts, equating to 99.2% differential
6 expression (Table 1). Differential expression of IL-1 β in the primary tumors of breast cancer patients
7 treated with trastuzumab was statistically significant (Table 1; $p=0.01869$).

8 We queried a second dataset⁴ to validate our findings of IL-1 β differential expression upon
9 trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with
10 trastuzumab, we again found that IL-1 β was among the genes whose expression was most significantly
11 different transcriptome-wide when comparing the global gene expression profiles of tumors from patients
12 treated with trastuzumab as compared to those not treated with trastuzumab. When sorting each of the
13 genes expressed in the tumors of patients treated with trastuzumab based on significance of change as
14 compared to the tumors of patients not treated with trastuzumab, IL-1 β ranked 2009 out

of 54675 total transcripts, equating to 96.3% differential expression. Differential expression of IL-1 β in the primary tumors of patients treated with trastuzumab was not deemed statistically significant but trended towards significance (Table 2; $p=0.089389$).

IL-1 β is expressed at higher levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for IL-1 β from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of IL-1 β change in the tumors of patients treated with trastuzumab. IL-1 β expression was significantly higher in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab (Figure 1). Increased expression of IL-1 β was statistically significant (Figure 1: $p=0.0127$). We calculated a mean fold change of 0.136928 when comparing the expression of IL-1 β in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that the pro-inflammatory cytokine IL-1 β was among the genes most differentially expressed in the primary tumors of patients with breast cancer and that IL-1 β expression was significantly higher in the primary tumors of patients treated with trastuzumab as compared to those not treated.

Discussion

IL-1 β can support invasive properties of MCF-7 breast cancer cells by activating gene expression of Src and the focal adhesion kinase¹¹, can promote the invasive properties of Lewis lung carcinoma (LLC) cells in syngeneic C57BL/6 mice by inducing expression of CXCL2 and the vascular endothelial growth factor¹², both of which function to support angiogenesis; tumors from LLC cells ectopically expressing IL-1 β grew 240% faster and displayed 250% more vasculature¹². Moreover, IL-1 β is associated with skeletal colonization in experimental models of prostate cancer, validated by analysis of Oncomine dataset⁹. Most importantly, in a cohort of over 1300 patients, tumor expression of IL-1 β was significantly correlated with relapse of disease to the bones and other sites⁸. In addition, over-expression of IL-1 β in three separate breast cancer cell lines, both estrogen receptor (ER)+ (MDA-MB-231) and ER- (T47D and MCF7) resulted in enhanced invasion and migration of breast cancer cells *in vivo*⁸.

We provided evidence here that the cytokine most classically associated with the canonical inflammasome, the pro-inflammatory IL-1 β , is expressed at significantly higher levels in the tumors of patients treated with trastuzumab and among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab.

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Rank	ID	p-value	t	B	FC	Gene
12	GI_27894305-S-1	0.01869	2.444482	-4.22	2.35 ± 2.03	IL1b

Table 1: IL-1 β 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 IL-1 β 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
2009	39402_at	0.089389	-1.72915592	-4.28	IL1B	interleukin 1 beta

Table 2: IL-1 β 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 IL-1 β 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: IL-1 β is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of IL-1 β 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 IL-1 β expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.