Repression of interleukin-8 in the primary tumors of breast cancer patients treated with trastuzumab.

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Cytokines are soluble factors that mediate signaling in the immune system¹. Immune control of tumor growth is a critical component of intrinsic anti-tumor defense². The cytokine interleukin-8 (IL-8) functions in activation and recruitment of neutrophils, innate immune cells with respiratory burst properties that also function as antigen-presenting cells^{3,4}. We found, using public and published microarray data^{5,6}, that the primary tumors of breast cancer patients treated with trastuzumab (Herceptin) express markedly lower quantities of IL-8 than those not treated with trastuzumab. These data allude to potential failures of innate and adaptive immune functions of the neutrophil in patients treated with trastuzumab.

Keywords: breast cancer, anti-tumor immunity, neutrophils, IL-8, interleukin-8, CXCL8, systems biology of breast cancer, targeted therapeutics in breast cancer.

1 The mechanism of action by which trastuzumab is thought to exert its function in breast cancer is
2 thought to involve binding of the Fab region of the trastuzumab antibody to the extracellular domain of
3 the human epidermal growth factor receptor 2 (HER2). The downstream consequences of such binding
4 events, including signals transduced and effectors functions affected by such signal transduction are less
5 well understood. We mined published and public microarray data to understand in an unbiased and
systematic fashion genes most transcriptionally perturbed following treatment of breast cancer patients
with trastuzumab. We identified significantly decreased expression of the cytokine interleukin-8 (also
known as CXCL8) in the primary tumors of breast cancer patients treated with trastuzumab.

6 **Methods**

7 We used datasets GSE38057⁵ and GSE130788⁶ for this global and multiplexed differential gene
8 expression analysis in conjunction with GEO2R. GSE38057 was generated using DASL Human Cancer
9 Panel by Probe technology with either $n=11$ primary breast tumors from patients treated not treated with
10 trastuzumab and $n=36$ primary breast tumors from patients treated trastuzumab; analysis was performed
11 using platform GPL5826. GSE130788 was generated using Whole Human Genome Microarray 4x44K
12 G4112F technology with $n=110$ primary breast tumors from patients before trastuzumab treatment and
13 $n=89$ primary tumors from patients after trastuzumab treatment, analysis performed using platform
14 GPL6480. The Benjamini and Hochberg method of p -value adjustment was used for ranking of
15 differential expression but raw p -values were used to assess statistical significance of global or
16 multiplexed differential expression. Log-transformation of data was auto-detected, and the NCBI
17 generated category of platform annotation was used. A statistical test was performed to evaluate whether
18 IL8 gene expression was significantly between primary breast tumors of patients receiving trastuzumab or
19 not 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).

16 **Results**

17 We performed a systems-level blind analysis of the transcriptional response to trastuzumab in
patients with breast cancer.

18 **IL-8 (CXCL8) is differentially expressed in the primary tumors of breast cancer patients treated 19 with trastuzumab.**

20 We identified the gene encoding interleukin-8, also known as CXCL8, among the genes most
21 whose expression changed most significantly when comparing the primary tumors of trastuzumab-treated
22 breast cancer patients to those not treated with trastuzumab, using a multiplexed gene expression dataset⁵.
23 When ranking each of the genes expressed in the primary tumors of trastuzumab-treated breast cancer
24 patients based on significance of change in expression as compared to that of patients not treated with
trastuzumab, IL-8 ranked 39 out of 1506 total transcripts (Table 1). Differential expression of IL-8 in the
tumors of trastuzumab-treated breast cancer patients was statistically significant (Table 1; $p=0.025213$).

25 We mined a second dataset, this a microarray dataset⁶, to compare whole transcriptome profiles of
26 primary tumors from trastuzumab-treated breast cancer patients to that of patients not treated with
trastuzumab. We were able to validate IL-8 as among the genes whose expression changed most
27 significantly when comparing global transcription from primary tumors of trastuzumab-treated patients as
28 compared to that of patients not treated with trastuzumab (Table 2). When ranking each of the genes
expressed in the primary tumors of trastuzumab-treated breast cancer patients based on significance of
change in expression as compared to that of patients not treated with trastuzumab, IL-8 ranked 9319 out

of 41000 total transcripts (Table 2), equating to 77.3% differential expression. Differential expression of IL-8 in the tumors of trastuzumab-treated breast cancer patients was statistically significant (Table 2; $p=7.83E-08$).

IL-8 is expressed at significantly lower levels in the primary tumors of breast cancer patients treated with trastuzumab as compared to those not treated with trastuzumab.

We obtained exact mRNA expression levels of IL-8 from the primary tumors of trastuzumab-treated breast cancer patients and from the primary tumors of breast cancer patients not treated with trastuzumab to directly compare IL-8 expression and determine the magnitude and direction of change in IL-8 expression following treatment with trastuzumab. We found that the primary tumors of trastuzumab-treated breast cancer patients expressed markedly lower levels of IL-8 as compared to the primary tumors of breast cancer patients not treated with trastuzumab (Figure 1). The difference in IL-8 mRNA expression between the primary tumors of trastuzumab-treated patients and patients not treated with trastuzumab treatment was statistically significant (Figure 1; $p=0.0485$).

Discussion

IL-8 is also known as the neutrophil chemotactic factor. IL-8 produced by cells of the monocyte/macrophage lineage can functionally activate neutrophils⁷. This has direct relevance to human cancer. One study demonstrated that neutrophil infiltration triggered by IL-8 could decrease tumorigenicity of human ovarian cancers⁸. Supporting the notion that neutrophil infiltration can confer anti-tumor properties is a study describing a positive relationship between patient prognosis and neutrophil infiltration in patients with colon cancer⁹. Such mechanisms and functions of the neutrophil have been described in human breast cancer, functioning through a NFAT signaling pathway¹⁰.

We provided evidence here that the primary tumors of breast cancer patients who had been treated with trastuzumab expressed significantly lower levels of IL-8, a cytokine with critical functions in the recruitment and activation of the neutrophil, a cell of the innate immune system with anti-tumor properties that also possesses adaptive functions. Decreased expression of IL-8 by the tumors of breast cancer patients resulting from treatment with trastuzumab may potentially translate to an immunocompromised state.

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Rank	ID	p-value	t	B	FC	Gene
39	GI_28610153-S-5	0.025213	-2.30942	-3.67	0.64 ± 0.50	IL8

Table 1: Differential expression of IL-8 (CXCL8) in the primary tumors of trastuzumab-treated breast cancer patients.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, fold change (FC) of IL-8 expression between the primary tumors of breast cancer patients treated with trastuzumab as compared to that of patients not treated with trastuzumab, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
9319	A_32_P87013	7.83E-08	-5.578661	6.88197	CXCL8	C-X-C motif chemokine ligand 8

Table 2: Differential expression of IL-8 (CXCL8) in the primary tumors of trastuzumab-treated breast cancer patients.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the primary tumors of breast cancer patients treated with trastuzumab as compared to that of patients not treated with trastuzumab, gene symbol and gene name are listed in this chart.

Figure 1: IL-8 (CXCL8) is expressed at significantly lower levels in the primary tumors of trastuzumab-treated breast cancer patients.

The mRNA expression level of the interleukin-8 cytokine 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) is graphically represented here with mean IL-8 levels marked and the result of a statistical test evaluating difference in mRNA expression level between patients treated with trastuzumab or not, a *p*-value, listed above.