Interleukin-4 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 and gene expression data^{3,4} to discover in an unbiased manner the most striking transcriptional features of trastuzumab treatment. We identified the cytokine interleukin-4 as among the genes most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab. Though IL-4 is most well known for its role as a cytokine supporting B-cell function through directing lineage commitment of type 2 T-helper lymphocytes^{5,6}, IL-4 can support tumor survival⁷. A single administration of trastuzumab was sufficient to drive differential expression of IL-4 in the primary tumors of patients with breast cancer, suggesting that IL-4 is directly transcriptionally induced by trastuzumab in patients with breast cancer. Thus, trastuzumab induces expression of a cytokine that fuels tumor growth through evasion of cell death⁷.

Keywords: trastuzumab, breast cancer, IL-4, systems biology of breast cancer, targeted therapeutics in breast cancer.

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

8 **Methods**

9 We used datasets GSE38057³ and GSE114082⁴ this differential gene expression analysis of the
10 transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was
11 generated using DASL Human Cancer Panel by Probe technology with *n*=6 primary breast tumors from
12 patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and *n*=35
13 primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in
14 addition to trastuzumab, both of which developed brain metastases; analysis was performed using
15 platform GPL5826. GSE114082 was generated using Illumina HumanHT-12 WG-DASL V4.0 R2
16 expression beadchip technology with *n*=17 primary breast tumors from patients before trastuzumab
17 treatment and *n*=17 primary tumors from patients after trastuzumab treatment, analysis performed using
18 platform GPL14951. The Benjamini and Hochberg method of *p*-value adjustment was used for ranking
19 of differential expression but raw *p*-values were used to assess statistical significance of global or
20 multiplexed differential expression. Log-transformation of data was auto-detected, and the NCBI
21 generated category of platform annotation was used. A statistical test was performed to evaluate whether
22 IL-4 gene expression was significantly between primary breast tumors of patients receiving trastuzumab
23 using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of
24 differential gene expression in human breast cancer (Version 8.4.0)(455).

25 **Results**

26 We harnessed the power of blind, comparative transcriptome analysis using published microarray
27 and gene expression data^{3,4} to identify the most significant transcriptional features of the primary breast
28 tumors of patients treated with trastuzumab (Herceptin).

29 **IL-4 is differentially expressed in the tumors of patients treated with trastuzumab.**

30 We identified the cytokine interleukin-4, IL-4, as among the genes whose expression changed
31 most significantly in the tumors of breast cancer patients treated with trastuzumab³ (Table 1). When
32 sorting each of the genes expressed in the primary tumors of breast cancer patients treated with
33 trastuzumab based on significance of change as compared to that of patients not treated with trastuzumab,
34 IL-4 ranked 11 out of 1506 total transcripts (Table 1), equating to 99.3% differential expression. IL-4
35 differential expression in the tumors of patients treated with trastuzumab was statistically significant
36 (Table 1; *p*=0.0183).

37 The first dataset, although measuring the expression of over 1500 genes, was still limited. We
38 turned to a second microarray dataset⁴, this measuring the expression of genes across the transcriptome -

29377 genes in total - but with another advantage. 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 differentially expressed genes when comparing tumors based on 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 treatment of trastuzumab, are more likely to be a direct transcriptional result of trastuzumab binding events. We found again that IL-4 was among the genes most differentially expressed in the tumors of patients administered a single treatment of trastuzumab (Table 2). When sorting each of the genes expressed in the tumors of patients administered a single treatment of trastuzumab, IL-4 ranked 2466 out of 29377 total transcripts (Table 2), equating to 91.6% differential expression. Differential expression of IL-4 in the primary tumors of breast cancer patients following treatment with trastuzumab was statistically significant (Table 2; $p=8.58E-04$).

IL-4 is expressed at significantly higher levels in the tumors of breast cancer patients treated with trastuzumab than in the tumors of those not treated with trastuzumab.

We obtained exact mRNA expression levels for IL-4 from tumors of patients treated with trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate magnitude and change of expression in IL-4 based on history of trastuzumab treatment. IL-4 was expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with trastuzumab (Figure 1; $p=0.0044$). We calculated a mean fold change of 1.43 ± 0.47 in IL-4 expression in the primary tumors of patients treated with trastuzumab relative to that of patients not treated with trastuzumab.

Thus, we found that IL-4 was among the genes most differentially expressed in the tumors of patients treated with trastuzumab, that IL-4 was expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab, and that this transcriptional change was likely a direct result of trastuzumab treatment as opposed to a gene expression change associated with tumor evolution.

Discussion

IL-4 directs differentiation of T-lymphocytes to the T-helper 2 lineage (T_H2), cells that produce IL-4, IL-5 and IL-10, and by guiding lymphocyte fate to the T_H2 lineage, supports the function of B-lymphocytes in immunoglobulin (antibody) production^{5,6}. However, IL-4 has a specific function in cancer wherein it can function to promote survival and growth of the tumor by supporting evasion of cell death through up-regulation of anti-apoptotic genes Bcl-x_L and cFLIP⁷.

We provided evidence here that the cytokine IL-4, a cytokine expressed at high levels in multiple tumor types, is a differentially expressed gene in the tumors of patients treated with trastuzumab; a single, brief exposure to trastuzumab was sufficient to drive expression of IL-4, indicating that trastuzumab directly transcriptionally induces expression of a cytokine that promotes rather than restricts tumor growth by supporting evasion of cell death through up-regulation of anti-apoptotic effectors.

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Rank	ID	p-value	t	B	FC	Gene
11	GI_27477091-A-3	0.0183	2.453057	-4.22	1.43 ± 0.47	IL4

Table 1: IL-4 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-4 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
2466	ILMN_2389080	8.58E-04	3.6376335	-0.73499	IL4

Table 2: A single, brief exposure to trastuzumab is sufficient to drive differential expression of IL-4 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: IL-4 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab

The mRNA expression level of IL-4 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-4 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.