The interleukin-23 receptor and a subunit for the IL-23 cytokine, IL-23A, are 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 and public microarray data³⁻⁶ to discover in an unbiased manner the most significant transcriptional changes associated with trastuzumab treatment. We identified the receptor for interleukin-23, IL23R, among the genes most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab^{3,4}. We also found that the alpha subunit of the IL-23 cytokine, IL23A, was among the genes whose expression changed most significantly in the primary tumors of patients treated with trastuzumab^{5,6}. IL23R messenger RNA was expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab than patients not treated with trastuzumab, demonstrating increased expression of the receptor for a cytokine that has been described to promote tumor growth⁷ as a transcriptional consequence associated with treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, IL23R, IL23, IL23A, 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 and gene expression data^{3,4}, we report here that IL23R and IL23A are differentially expressed genes in the tumors of trastuzumab-treated patients: patients treated with trastuzumab expressed significantly higher levels of IL23R than did those of patients not treated with trastuzumab.

Methods

We used datasets GSE114082³, GSE130788⁴, GSE75685⁵, and GSE50948⁶ for this differential gene expression analysis of the transcriptional response to trastuzumab treatment in patients with breast cancer. 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. 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. GSE50948 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with *n*=93 primary tumors of the breast from patients not treated with trastuzumab and *n*=63 primary tumors of the breast from patients treated with trastuzumab; platform GPL570 was utilized for analysis. GSE75685 was generated using Whole Human Genome Microarray 4x44K technology with *n*=41 primary breast tumors from patients not treated with trastuzumab and *n*=11 primary tumors from patients treated with trastuzumab, analysis performed using platform GPL4133. 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 IL23R 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 and gene expression data^{3,4} to identify the most significant transcriptional features of the primary breast tumors of patients treated with trastuzumab (Herceptin).

IL23R is the single most differentially expressed gene in the tumors of patients treated with trastuzumab.

We identified the IL-23 receptor encoded by IL23R as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab in a microarray dataset³ (Table 1). 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. When sorting each of the genes expressed in the primary tumors of breast cancer patients treated with a single dose of trastuzumab based on significance of change as compared tumors from the same patients before treatment with trastuzumab, IL23R ranked 1 out of 29377 total transcripts (Table 1), equating to 99.997% differential expression. IL23R differential expression in the tumors of patients treated with trastuzumab was statistically significant (Table 1; $p=1.22E-09$).

We validated statistically significant transcriptome-wide differential expression of IL-23R in the tumors of patients treated with trastuzumab in a second microarray dataset. When sorting each of the genes expressed in the tumors of patients treated with trastuzumab based on significance of change in expression as compared to the tumors of patients not treated with trastuzumab, IL23R ranked 20370 out of 41000 total transcripts (Table 2), equating to 49.7% differential expression. Differential expression of IL23R in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 2; $p=3.29E-03$).

IL23A is differentially expressed in the tumors of patients treated with trastuzumab.

Analysis of a separate microarray dataset revealed that the alpha subunit of the IL-23 cytokine, IL23Am was among the genes most differentially expressed in the tumors of patients treated with trastuzumab when compared to patients not treated with trastuzumab (Table 2). When sorting each of the genes expressed in the tumors of patients treated with trastuzumab based on significance of change in expression as compared to the tumors of patients not treated with trastuzumab, IL23A ranked 1282 out of 54675 total transcripts (Table 3), equating to 97.7% differential expression. Differential expression of IL23A in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 3; $p=2.12E-02$).

We queried a fourth microarray dataset to validate differential expression of IL-23A in the tumors of patients treated with trastuzumab. We found in this case that IL-23A expression changed more significantly than 88.3% of the measured transcriptome, but not to a statistically significant extent. When sorting each of the genes expressed in the tumors of patients treated with trastuzumab based on significance of change in expression as compared to the tumors of patients not treated with trastuzumab, IL23A ranked 5302 out of 45220 total transcripts (Table 4), equating to 88.3% differential expression. Differential expression of IL23A in the primary tumors of breast cancer patients treated with trastuzumab was not deemed statistically significant (Table 4; $p=1.94E-02$).

IL23R 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 IL23R from tumors of patients treated with trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate direction and statistical significance of change in expression of IL23R following a brief exposure to trastuzumab. IL23R was expressed at higher levels in the tumors of patients after receiving a single dose of trastuzumab, and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 11.58 ± 8.99 in the tumors of patients treated with a single dose of trastuzumab as compared to tumors from the same patients before treatment with trastuzumab. A single dose of trastuzumab was sufficient to result in nearly 12-fold induction of the IL-23 receptor in primary tumors of the breast.

1 Thus, we found using published microarray data³ that IL23R was among the genes most
2 differentially expressed in the tumors of patients treated with trastuzumab, that IL23R was expressed at
3 higher levels in the tumors of patients following administration of a single dose of trastuzumab. A
4 separate microarray dataset revealed the IL-23 alpha subunit, IL-23A was among the genes most
5 differentially expressed in the tumors of patients treated with trastuzumab. We can conclude that
transcriptional induction of IL-23R is a direct transcriptional result of treatment with trastuzumab
treatment as opposed to a gene expression change associated with clonal selection and evolution of the
tumor across the course of treatment.

6 Discussion

7 We provided evidence here that IL23R is a differentially expressed gene in the tumors of patients
8 treated with trastuzumab, and expressed at significantly higher levels in the primary tumors of breast
9 cancer patients following a single dose of trastuzumab. In a separate dataset, Thus, trastuzumab, a
10 treatment whose use in patients with breast cancer has been associated with a 34% rate of central nervous
11 system metastases⁸, results in increased expression of the receptor for a cytokine that promotes both the
12 incidence and growth of tumors⁷; a subunit of that cytokine itself is also differentially expressed when
13 comparing the tumors of patients treated with trastuzumab with those not treated with trastuzumab. Thus,
14 modulation of the gene expression of IL-23 and the IL-23 receptor appear to be significant transcriptional
15 events associated with trastuzumab treatment in the tumors of breast cancer patients treated with
16 trastuzumab, and literature⁷ suggests that these changes are not chemotherapeutic.
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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. 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.
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>. Hsiaowang Chen. UCLA, Department of Medicine. Santa Monica, CA.
5. Prat, A., Bianchini, G., Thomas, M., Belousov, A., Cheang, M.C., Koehler, A., Gómez, P., Semiglazov, V., Eiermann, W., Tjulandin, S. and Byakhov, M., 2014. based PAM50 subtype predictor identifies higher responses and improved survival outcomes in HER2-positive breast cancer in the NOAH study. *Clinical Cancer Research*, 20(2), pp.511-521.
6. GSE75685. Mexican Patients with Breast Cancer. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE75685>. Hugo Gomez, Bioinformatics, ITESM, Mexico.
7. Langowski, J.L., Zhang, X., Wu, L., Mattson, J.D., Chen, T., Smith, K., Basham, B., McClanahan, T., Kastelein, R.A. and Oft, M., 2006. IL-23 promotes tumour incidence and growth. *Nature*, 442(7101), pp.461-465.
8. Bendell, J.C., Domchek, S.M., Burstein, H.J., Harris, L., Younger, J., Kuter, I., Bunnell, C., Rue, M., Gelman, R. and Winer, E., 2003. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer*, 97(12), pp.2972-2977.

Rank	ID	p-value	t	B	FC	Gene
1	ILMN_2223663	1.22E-09	8.1189824	11.83781	11.58 ± 8.99	IL23R

Table 1: IL23R is the single most differentially expressed gene in the primary tumors of breast cancer patients following treatment 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, the fold change of IL23R gene expression in the tumors of patients treated with trastuzumab as compared to tumors from the same patients before treatment with trastuzumab, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
20370	A_23_P425197	3.29E-03	-2.975651	-3.269971	IL23R	interleukin 23 receptor

Table 2: IL23R 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	Gene name
1282	234865_at	2.12E-02	2.3285076	-3.3111	IL23A	interleukin 23 subunit alpha

Table 3: IL-23A is differentially expressed in the primary tumors of patients with breast cancer that are 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	Gene name
5302	20211	1.94E-02	2.4129427	-3.395713	IL23R	interleukin 23 receptor

Table 4: IL-23A is differentially expressed in the primary tumors of patients with breast cancer that are treated with trastuzumab, but in this dataset, not to a statistically significant extent.

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: IL23R is transcriptionally induced at a significant level in the primary tumors of patients following a single dose of trastuzumab.

The mRNA expression level of IL23R 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 IL23R expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.