SYK, CSK and FYN are differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

The mechanism of action underlying trastuzumab (Herceptin) function is ascribed to binding of the F_{ab} region of trastuzumab to the extracellular domain of the human epidermal growth factor receptor (HER2)¹. The transcriptional responses that follow signals transduced after trastuzumab binding of HER2 are less well understood. Protein tyrosine kinases are one major class of cellular components utilized in the transduction of signals from the cell surface to the nucleus. We mined published microarray and multiplexed gene expression data²⁻⁵ to understand in an unbiased fashion genes most differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab. We observed significantly increased and differential expression of multiple protein tyrosine kinases in the primary tumors of patients with breast cancer, including SYK, CSK and FYN. These data document previously undescribed up-regulation of protein tyrosine kinases following treatment with trastuzumab in patients with breast cancer.

Keywords: trastuzumab, breast cancer, SYK, CSK, FYN, tyrosine kinases in cancer, systems biology of breast cancer, targeted therapeutics in breast cancer.

Trastuzumab (Herceptin), a monoclonal antibody utilized for the treatment of adjuvant and metastatic breast cancer in the United States, targets the human epidermal growth factor receptor, or HER2^{1,8}. How gene expression is most significantly different between the tumors of breast cancer patients treated with trastuzumab and those not treated with trastuzumab has not been fully explored. We mined published microarray and multiplexed datasets²⁻⁵ to understand in an unbiased fashion and at the systems level genes most transcriptionally perturbed by treatment with trastuzumab in the primary tumors of patients with breast cancer. We found significant differential expression of the genes encoding three protein tyrosine kinases in the primary tumors of patients treated with trastuzumab: SYK, CSK, and FYN.

Methods

We used datasets GSE38057², GSE66399³, GSE22226⁴, and GSE130788⁵ and for 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 $n=6$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=35$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in addition to trastuzumab, both of which had developed brain metastases (SYK and CSK); or $n=11$ patients not treated with trastuzumab and $n=70$ patients treated with trastuzumab (FYN), some of which had developed brain metastases and some of which had not; both analyses performed using platform GPL5826. GSE66399 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=31$ primary breast tumors from patients treated with trastuzumab and $n=23$ primary tumors from patients treated with trastuzumab, analysis performed using platform GPL570. GSE22226 was generated using Whole Human Genome Oligo Microarray G4112A technology with $n=118$ primary breast tumors from patients treated with chemotherapy and $n=11$ primary tumors from patients treated with chemotherapy and trastuzumab, analysis performed using platform GPL1708. 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 SYK, FYN or CSK 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 systems-level transcriptome analysis using published microarray and multiplexed gene expression data²⁻⁵, comparing primary tumors of breast cancer patients who had been treated or not with trastuzumab to describe in an unbiased fashion the most significant features of the transcriptional response to trastuzumab treatment in primary of the breast in humans with breast cancer.

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

We found that the protein tyrosine kinase SYK was among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab².

When sorting each of the genes expressed in trastuzumab-treated breast tumors based on significance of difference in expression as compared to the primary breast tumors of patients not treated with trastuzumab, SYK ranked 29 out of 1506 total transcripts, equating to 98.1% differential expression (Table 1). Differential expression of SYK in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.03118$).

We queried a second dataset to validate our findings of SYK differential expression upon trastuzumab treatment. We again found that SYK was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of primary breast 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 breast cancer patients treated with trastuzumab based on significance of change as compared to the tumors of patients not treated with trastuzumab, SYK ranked 189 out of 54675 total transcripts (Table 2), equating to 99.7% differential expression. Differential expression of SYK was statistically significant (Table 2; $p=0.015757$).

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

We identified a second protein tyrosine kinase, CSK, among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab². When sorting each of the genes expressed in trastuzumab-treated breast tumors based on significance of difference in expression as compared to the primary breast tumors of patients not treated with trastuzumab, CSK ranked 28 out of 1506 total transcripts (Table 3), equating to 98.1% differential expression. Differential expression of CSK in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 3; $p=0.02988$).

We queried a second dataset to validate our findings of CSK differential expression upon trastuzumab treatment. We again found that CSK was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of primary breast 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 breast cancer patients treated with trastuzumab based on significance of change as compared to the tumors of patients not treated with trastuzumab, CSK ranked 2763 out of 44290 total transcripts (Table 4), equating to 93.8% differential expression. Differential expression of CSK was statistically significant (Table 4; $p=3.49E-02$).

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

We identified a third protein tyrosine kinase, FYN, among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab². When sorting each of the genes expressed in trastuzumab-treated breast tumors based on significance of difference in expression as compared to the primary breast tumors of patients not treated with trastuzumab, FYN ranked 5 out of 1506 total transcripts (Table 5), equating to 99.7% differential expression. Differential expression of FYN in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 5; $p=0.009813$).

We queried a second dataset to validate our findings of FYN differential expression upon trastuzumab treatment. We again found that FYN was among the genes whose expression was most significantly different transcriptome-wide when comparing the global gene expression profiles of primary breast 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 breast cancer patients treated with trastuzumab

1 based on significance of change as compared to the tumors of patients not treated with trastuzumab, FYN
2 ranked 3618 out of 41000 total transcripts, equating to 91.2% differential expression (Table 6).
3 Differential expression of FYN was statistically significant (Table 6; $p=9.44E-15$).

4 **SYK, CSK and FYN are all expressed at higher levels in the primary tumors of patients treated**
5 **with trastuzumab as compared to that of patients not treated with trastuzumab.**

6 We obtained exact mRNA expression levels for SYK, CSK and FYN from the tumors of patients
7 treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine
8 magnitude and direction of protein tyrosine kinase change in the tumors of patients treated with
9 trastuzumab. SYK, CSK and FYN expression was higher in the tumors of patients treated with
10 trastuzumab as compared to the tumors of patients not treated with trastuzumab (Figures 1, 2 and 3,
11 respectively). We calculated a mean fold change of 1.45 ± 0.68 when comparing the expression of SYK
12 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with
13 trastuzumab. Increased expression of SYK was not deemed statistically significant (Figure 1: $p=0.1920$).
14 We calculated a mean fold change of 1.44 ± 0.49 when comparing the expression of CSK in the tumors of
15 patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.
16 Increased expression of CSK trended towards statistical significance (Figure 2: $p=0.0658$). We calculated
17 a mean fold change of 1.21 ± 0.32 when comparing the expression of FYN in the tumors of patients
18 treated with trastuzumab as compared to the tumors of patients treated with trastuzumab. Increased
19 expression of FYN was statistically significant (Figure 3: $p=0.0203$).

20 Thus, we found that three protein tyrosine kinases, SYK, CSK and FYN were among the most
21 significant transcriptional features of primary tumors in breast cancer patients treated with trastuzumab;
22 SYK, CSK and FYN were each present at higher quantities at the mRNA level in the primary tumors of
23 breast cancer patients treated with trastuzumab.

24 **Discussion**

25 The general paradigm associated with transduction of signals is that protein tyrosine kinase
26 receptors positively transduce signals, and this implies that an increased level of signaling components
27 will translate to increased signal transduction. The end result of signals transduced across the membrane
28 will vary depending on cell-type specific expression of regulatory components, post-translational
modification of pathway machinery and how this translates into integration of a final cellular output
resulting from modulation of gene expression. However, it is difficult to envision how the concurrent up-
regulation of multiple protein tyrosine kinases would be chemotherapeutic in any sense.

We cannot rule out the possibility that these results reflect pervasive contamination of tissue
samples with blood cells, that trastuzumab treatment affects signaling at the T-cell receptor and that this is
what we are observing in the microarray datasets we analyzed. However, it is more likely these reflect
changes in gene expression of central cell signaling components inherent to primary breast tumors
induced over the course of treatment with trastuzumab in humans with breast cancer.

We provided evidence here that multiple protein tyrosine kinases - SYK, CSK and FYN - are
among the genes whose expression changes most significantly transcriptome-wide in the primary tumors
of patients with breast cancer upon treatment with trastuzumab, and that these protein tyrosine kinases are
all expressed at increased quantities in the tumors of patients with breast cancer treated with trastuzumab.

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. 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.
3. Guarneri, V., Dieci, M.V., Frassoldati, A., Maiorana, A., Ficarra, G., Bettelli, S., Tagliafico, E., Biccato, S., Generali, D.G., Cagossi, K. and Bisagni, G., 2015. Prospective biomarker analysis of the randomized CHER-LOB study evaluating the dual anti-HER2 treatment with trastuzumab and lapatinib plus chemotherapy as neoadjuvant therapy for HER2-positive breast cancer. *The oncologist*, 20(9), p.1001.
4. Esserman, L.J., Berry, D.A., Cheang, M.C., Yau, C., Perou, C.M., Carey, L., DeMichele, A., Gray, J.W., Conway-Dorsey, K., Lenburg, M.E. and Buxton, M.B., 2012. Chemotherapy response and recurrence-free survival in neoadjuvant breast cancer depends on biomarker profiles: results from the I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657). *Breast cancer research and treatment*, 132(3), pp.1049-1062.
5. 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.
6. Fluck, M., Zurcher, G., Andres, A.C. and Ziemiecki, A., 1995. Molecular characterization of the murine syk protein tyrosine kinase cDNA, transcripts and protein. *Biochemical and biophysical research communications*, 213(1), pp.273-281.
7. Chow, L.M., Fournel, M., Davidson, D. and Veillette, A., 1993. Negative regulation of T-cell receptor signalling by tyrosine protein kinase p50 csk. *Nature*, 365(6442), pp.156-160.
8. Samelson, L.E., Phillips, A.F., Luong, E.T. and Klausner, R.D., 1990. Association of the fyn protein-tyrosine kinase with the T-cell antigen receptor. *Proceedings of the National Academy of Sciences*, 87(11), pp.4358-4362.

Rank	ID	p-value	t	B	FC	Gene
29	GI_34147655-S-1	0.03118	2.227906	-4.29	1.45 ± 0.68	SYK

Table 1: SYK 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 SYK 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
189	207540_s_at	0.015757	-2.49168874	-3.74	SYK	spleen associated tyrosine kinase

Table 2: SYK 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	FC	Gene
28	GI_4758077-S-2	0.02988	2.246315	-4.29	1.44 ± 0.49	NM_004383.1

Table 3: CSK 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 CSK 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
2763	10911	3.49E-02	-2.13	-3.67855	CSK	c-src tyrosine kinase

Table 4: CSK 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	FC	Gene
5	GI_23510363-A-3	0.009813	2.639253	-3.61	1.21 ± 0.32	FYN

Table 5: FYN 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 FYN 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
3618	A_32_P224888	9.44E-15	-8.37951	22.532167	FYN	FYN proto-oncogene, Src family tyrosine kinase

Table 6: FYN 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.

Figure 1: SYK 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 SYK 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 SYK expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.

Figure 2: CSK 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 CSK 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 CSK expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.

Figure 3: FYN 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 FYN 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 FYN expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.