Src tyrosine kinase is differentially expressed in the tumors of breast cancer patients treated with trastuzumab.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Trastuzumab (Herceptin), a monoclonal antibody targeting the extracellular domain of the human epidermal growth factor receptor 2 (HER2)¹, is 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 fashion the most significant transcriptional changes associated with treatment with trastuzumab in patients with breast cancer. We identified the proto-oncogene and tyrosine kinase Src as among the genes most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab. The primary tumors of breast cancer patients treated with trastuzumab expressed higher levels of Src messenger than did patients not treated with trastuzumab, and a single administration of trastuzumab was sufficient to result in differential expression of Src in primary tumors of the breast, demonstrating that a proto-oncogene⁵ and gene whose product is necessary to drive MMTV-induced tumorigenesis of the mammary gland in mice⁶ is transcriptionally induced in primary tumors of the breast, likely as a direct result of treatment with trastuzumab.

Keywords: trastuzumab, breast cancer, Src, 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. By performing unbiased, systems-level comparative transcriptome analysis of primary tumors of the breast in patients treated with trastuzumab and those not treated with trastuzumab using published microarray and gene expression data^{3,4}, we report here that the tyrosine kinase Src is a differentially expressed gene in the tumors of trastuzumab-treated patients: the primary tumors of breast cancer patients treated with trastuzumab expressed higher levels of Src than did those of patients not treated with trastuzumab.

Methods

We used datasets GSE38057³ and GSE114082⁴ this 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 developed brain metastases; analysis was performed using platform GPL5826. 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. 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 Src 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 harnessed the power of blind, 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).

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

We identified the transcription factor Src as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab in a multiplexed gene expression dataset³ (Table 1). When sorting each of the genes expressed in the primary tumors of breast cancer patients treated with trastuzumab based on significance of change as compared to that of patients not treated with trastuzumab, Src ranked 60 out of 1506 total transcripts (Table 1), equating to 96.0% differential expression. Src differential expression in the tumors of patients treated with trastuzumab was statistically significant (Table 1; $p=0.05742$).

The first dataset, although measuring the expression of over 1500 genes, was still limited. We turned to a second microarray dataset⁴, this measuring the expression of genes across the transcriptome - 29377 genes in total. The tumors compared in this dataset were sampled from the same patient before and after a single 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

1 resulting from tumor evolution over the course of treatment, changes identified in this dataset, occurring
2 after only a single treatment of trastuzumab, are more likely to be a direct transcriptional result of
3 trastuzumab binding events. We found again that Src was among the genes most differentially expressed
4 in the tumors of patients administered a single treatment of trastuzumab (Table 2). When sorting each of
5 the genes expressed in the tumors of patients administered a single treatment of trastuzumab, Src ranked
6 4614 out of 29377 total transcripts (Table 2), equating to 84.3% differential expression. Differential
7 expression of Src in the primary tumors of breast cancer patients following treatment with trastuzumab
8 was statistically significant (Table 2; $p=5E-03$).

9 **Src is expressed at higher levels in the tumors of breast cancer patients treated with**
10 **trastuzumab than in the tumors of those not treated with trastuzumab.**

11 We obtained exact mRNA expression levels for Src from tumors of patients treated with
12 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate magnitude
13 and change of expression in Src based on history of trastuzumab treatment. Src was expressed at higher
14 levels in the tumors of patients treated with trastuzumab as compared to those of patients not treated with
15 trastuzumab, but this difference was not deemed statistically significant (Figure 1; $p=0.1502$). We
16 calculated a mean fold change of 1.45 ± 0.68 in Src expression in the primary tumors of patients treated
17 with trastuzumab relative to that of patients not treated with trastuzumab.

18 Thus, we found using published multiplexed gene expression data³ and a microarray dataset⁴ that
19 Src was among the genes most differentially expressed in the tumors of patients treated with trastuzumab,
20 that Src was expressed at higher levels in the tumors of patients treated with trastuzumab as compared to
21 those not treated with trastuzumab, and that this transcriptional change was likely a direct result of
22 trastuzumab treatment as opposed to a gene expression change associated with clonal selection and
23 evolution of the tumor across the course of treatment.

24 **Discussion**

25 There exist at least one dozen studies in the scientific literature that demonstrate the pro-
26 oncogenic and transformative properties of the Src tyrosine kinase^{6-7,9-18}. Of concern, Src can interact
27 with HER2, the molecular target of trastuzumab; we previously demonstrated that trastuzumab results in
28 transcriptional induction of ERBB2⁷, the gene that encodes HER2⁸. Thus, it appears that trastuzumab
may activate a transcriptional loop wherein treatment with trastuzumab results in induction of the gene
product which it targets, as well as a proto-oncogenic signaling molecule (Src) that supports signaling
through that gene product (HER2). Consistent with this, murine mammary tumors that express HER2
exhibit elevated Src kinase activity⁶. Src can suppress cell death (apoptosis) by phosphorylation of
caspase-8⁹. Aside from its capacity to support cell proliferation through mediation of signal transduction
using its kinase activity, Src can function to enhance cell spreading behaviors of cancer cells¹⁰ on
fibronectin independent of its kinase domain¹¹. Src can support metastasis to the bone in animal models
of breast cancer^{14,15}, and inhibiting Src kinase can inhibit metastasis of breast cancer cells in mice^{16,17}.
Src can function to support proliferation of breast cancer cells through STAT3¹⁸; we recently
demonstrated that trastuzumab is associated with increased expression of STAT3 as well¹⁹. Src inhibition
with saracatinib results in improvements in overall survival which may be due to decreased expression of
Myc and the glucose transported GLUT1²⁰.

26 We provided evidence here that Src, a tyrosine kinase and proto-oncogene is a differentially
27 expressed gene in the tumors of patients treated with trastuzumab, and that the primary tumors of breast
28 cancer patients treated with trastuzumab expressed higher levels of Src in their primary tumors than did
patients not treated with trastuzumab, likely a direct result of trastuzumab administration. Thus,
trastuzumab treatment results in or is associated with increased expression of SRC, STAT3, and HER2,
potentially activating a signal transduction pathway whose function it is to support rather than suppress
tumor cell proliferation.

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. 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.
4. 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.
5. Ralston, R. and Bishop, J.M., 1985. The product of the protooncogene c-src is modified during the cellular response to platelet-derived growth factor. *Proceedings of the National Academy of Sciences*, 82(23), pp.7845-7849.
6. Muthuswamy, S.K., Siegel, P.M., Dankort, D.L., Webster, M.A. and Muller, W.J., 1994. Mammary tumors expressing the neu proto-oncogene possess elevated c-Src tyrosine kinase activity. *Molecular and cellular biology*, 14(1), pp.735-743.
7. Belsches-Jablonski, A.P., Biscardi, J.S., Peavy, D.R., Tice, D.A., Romney, D.A. and Parsons, S.J., 2001. Src family kinases and HER2 interactions in human breast cancer cell growth and survival. *Oncogene*, 20(12), pp.1465-1475.
8. Mamoor, S., 2020. ERBB2 is differentially expressed in the tumors of breast cancer patients treated with trastuzumab, a monoclonal antibody targeting HER2, but ERBB2 expression increases rather than decreasing.
9. Allgayer, H., Boyd, D.D., Heiss, M.M., Abdalla, E.K., Curley, S.A. and Gallick, G.E., 2002. Activation of Src kinase in primary colorectal carcinoma: an indicator of poor clinical prognosis. *Cancer*, 94(2), pp.344-351.
10. Cursi, S., Rufini, A., Stagni, V., Condò, I., Matafora, V., Bachi, A., Bonifazi, A.P., Coppola, L., Superti-Furga, G., Testi, R. and Barilà, D., 2006. Src kinase phosphorylates Caspase-8 on Tyr380: a novel mechanism of apoptosis suppression. *The EMBO journal*, 25(9), pp.1895-1905.
11. Boyer, B., Bourgeois, Y. and Poupon, M.F., 2002. Src kinase contributes to the metastatic spread of carcinoma cells. *Oncogene*, 21(15), pp.2347-2356.
12. Kaplan, K.B., Swedlow, J.R., Morgan, D.O. and Varmus, H.E., 1995. c-Src enhances the spreading of src-/-fibroblasts on fibronectin by a kinase-independent mechanism. *Genes & development*, 9(12), pp. 1505-1517.

13. Elsberger, B., Fullerton, R., Zino, S., Jordan, F., Mitchell, T.J., Brunton, V.G., Mallon, E.A., Shiels, P.G. and Edwards, J., 2010. Breast cancer patients' clinical outcome measures are associated with Src kinase family member expression. *British journal of cancer*, 103(6), pp.899-909.
14. Zhang, X.H.F., Wang, Q., Gerald, W., Hudis, C.A., Norton, L., Smid, M., Foekens, J.A. and Massagué, J., 2009. Latent bone metastasis in breast cancer tied to Src-dependent survival signals. *Cancer cell*, 16(1), pp.67-78.
15. Myoui, A., Nishimura, R., Williams, P.J., Hiraga, T., Tamura, D., Michigami, T., Mundy, G.R. and Yoneda, T., 2003. C-SRC tyrosine kinase activity is associated with tumor colonization in bone and lung in an animal model of human breast cancer metastasis. *Cancer research*, 63(16), pp.5028-5033.
16. Jallal, H., Valentino, M.L., Chen, G., Boschelli, F., Ali, S. and Rabbani, S.A., 2007. A Src/Abl kinase inhibitor, SKI-606, blocks breast cancer invasion, growth, and metastasis in vitro and in vivo. *Cancer Research*, 67(4), pp.1580-1588.
17. Rucci, N., Recchia, I., Angelucci, A., Alamanou, M., Del Fattore, A., Fortunati, D., Šuš, M., Fabbro, D., Bologna, M. and Teti, A., 2006. Inhibition of protein kinase c-Src reduces the incidence of breast cancer metastases and increases survival in mice: implications for therapy. *Journal of Pharmacology and Experimental Therapeutics*, 318(1), pp.161-172.
18. Garcia, R., Bowman, T.L., Niu, G., Yu, H., Minton, S., Muro-Cacho, C.A., Cox, C.E., Falcone, R., Fairclough, R., Parsons, S. and Laudano, A., 2001. Constitutive activation of Stat3 by the Src and JAK tyrosine kinases participates in growth regulation of human breast carcinoma cells. *Oncogene*, 20(20), pp.2499-2513.
19. Mamoor, S., 2020. Trastuzumab administration in patients with breast cancer is associated with increased primary tumor expression of STAT3.
20. Jain, S., Wang, X., Chang, C.C., Ibarra-Drendall, C., Wang, H., Zhang, Q., Brady, S.W., Li, P., Zhao, H., Dobbs, J. and Kyrish, M., 2015. Src inhibition blocks c-Myc translation and glucose metabolism to prevent the development of breast cancer. *Cancer research*, 75(22), pp.4863-4875.

Rank	ID	p-value	t	B	FC	Gene
60	GI_38202216-A-4	0.05742	1.9525	-4.38	1.45 ± 0.68	SRC

Table 1: Src 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 Src 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
4614	ILMN_1778253	5E-03	-2.9912582	-2.34603	SRC

Table 2: A single exposure to trastuzumab is sufficient to drive differential expression of Src 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: Src is expressed at higher levels in the primary tumors of patients treated with trastuzumab.

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