Trastuzumab treatment in patients with breast cancer is associated with differential expression of the Wilms' tumor susceptibility gene, WT1.

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 through trastuzumab binding to HER2 are less well understood. We mined published microarray and multiplexed gene expression data^{2,3} 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 the Wilms' tumor susceptibility gene, WT1.

Keywords: trastuzumab, breast cancer, WT1, 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^{1,6}. How transcription is most significantly different between the tumors of breast cancer patients treated with trastuzumab and those not treated with trastuzumab is not fully explored. We mined published microarray and multiplexed datasets^{2,3} 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 gene encoding the Wilms' tumor susceptibility gene, WT1^{4,5}.

Methods

We used datasets GSE38057² and GSE114082³ 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 either $n=11$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=36$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in addition to trastuzumab; 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 WT1 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 data^{2,3}, 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 tumors from humans with breast cancer.

WT1 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that the Wilms' tumor susceptibility gene, WT1 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, WT1 ranked 18 out of 1506 total transcripts, equating to 98.8% statistical significance (Table 1). Differential expression of WT1 in the primary tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 1; $p=0.013526$).

We queried a second microarray dataset³ which utilized tumor tissue samples of the same patient before and after a single treatment with trastuzumab, as if WT1 was differentially expressed in this it would likely be a direct transcriptional consequence of trastuzumab treatment. WT1 was again among

the genes most differentially expressed, albeit to a lesser degree in this dataset (Table 1). When sorting each of the genes expressed in the tumors of patients treated with trastuzumab based on significance of change as compared to the tumors of patients not treated with trastuzumab, WT1 ranked 8485 out of 29377 total transcripts (Table 2), equating to 71.1% differential expression. Differential expression of WT1 was statistically significant (Table 2; $p=3.21\text{E-}02$). Since these tissue samples were not comparing tumors from patients receiving trastuzumab or not, but were comparing tumors from the same individuals before and after a single treatment with trastuzumab, this indicates that induction of WT1 is likely a direct consequence of signals transduced downstream of trastuzumab binding to HER2, or of retrograde signal transduction transmitted when the F_c domain of trastuzumab engages one or more F_c receptors of unknown identity⁷.

WT1 is expressed at significantly higher levels in the primary tumors of patients treated with trastuzumab as compared to that of patients not treated with trastuzumab.

We obtained exact mRNA expression levels for WT1 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of WT1 change in the tumors of patients treated with trastuzumab. WT1 expression was significantly higher in the tumors of patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab (Figure 1). Increased expression of WT1 was statistically significant (Figure 1: $p=0.0144$). We calculated a mean fold change of 2.28 ± 2.14 when comparing the expression of WT1 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found through unbiased transcriptome analysis of primary tumors of patients with breast cancer that WT1 was among the most significant transcriptional features of primary tumors in patients treated with trastuzumab; WT1 was expressed at significantly increased levels in the primary tumors of patients treated with trastuzumab, and this may likely be a direct result of transcriptional modulation downstream of trastuzumab binding events.

Discussion

The locus for the Wilms' tumor gene product (WT1) is located on chromosome 11, and expression of the Wilms' tumor gene product could be observed most abundantly in the kidneys but also in cells of the hematopoietic system⁴. WT1 encodes for a protein containing four zinc finger domains and a proline, glutamine rich-region⁴. A major function of the Wilms' tumor gene product is in transcriptional repression⁵, including the repression of the insulin-like growth factor II (IGF-2)⁸, Bcl-2⁹ and c-Myc⁹. WT1 can also function in transcriptional activation (e.g., amphiregulin)¹⁰. One tissue-specific developmental function of WT1 is in the development of the retina; the retina of Wt1-deficient embryos is thinner than that of wild-type mouse embryos¹¹. A study of WT1 expression in the normal and cancerous breast demonstrated that WT1 expression could be detected in the mammary duct and lobule of the normal breast; out of 21 total primary tumors of the breast surveyed by immunohistochemistry for WT1 expression, 40% were classified as lacking immunodetectable WT1 and another 28% were classified as negative for WT1 expression¹². This, WT1 is zinc finger-containing molecule with most abundant expression in the kidneys involved in both repression and activation of genes, including the activation of amphiregulin, a gene with functions in epithelial branching.

In summary, we found here that the gene encoding the Wilms' tumor susceptibility gene WT1, a molecule that functions in both transcriptional repression and activation of target genes, is among the genes whose expression most significantly distinguishes the primary tumors of patients treated with trastuzumab; WT1 is likely directly transcriptionally induced by treatment with trastuzumab. While WT1 functions in the repression of multiple genes important for cancer cell survival and growth, like that of c-MYC and IGF-2.

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-Arlukowicz, 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. 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. Call, K.M., Glaser, T., Ito, C.Y., Buckler, A.J., Pelletier, J., Haber, D.A., Rose, E.A., Kral, A., Yeger, H., Lewis, W.H. and Jones, C., 1990. Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. *Cell*, 60(3), pp.509-520.
5. Madden, S.L., Cook, D.M., Morris, J.F., Gashler, A., Sukhatme, V.P. and Rauscher, F.J., 1991. Transcriptional repression mediated by the WT1 Wilms tumor gene product. *Science*, 253(5027), pp. 1550-1553.
6. Treating HER2+ Cancer. <https://www.herceptin.com/hcp/treating-HER2-cancer.html>.
7. Shi, Y., Fan, X., Deng, H., Brezski, R.J., Ryczyn, M., Jordan, R.E., Strohl, W.R., Zou, Q., Zhang, N. and An, Z., 2015. Trastuzumab triggers phagocytic killing of high HER2 cancer cells in vitro and in vivo by interaction with Fcγ receptors on macrophages. *The Journal of Immunology*, 194(9), pp. 4379-4386.
8. Drummond, I.A., Madden, S.L., Rohwer-Nutter, P., Bell, G.I., Sukhatme, V.P. and Rauscher, F.J., 1992. Repression of the insulin-like growth factor II gene by the Wilms tumor suppressor WT1. *Science*, 257(5070), pp.674-678.
9. Lee, S.B., Huang, K., Palmer, R., Truong, V.B., Herzlinger, D., Kolquist, K.A., Wong, J., Paulding, C., Yoon, S.K., Gerald, W. and Oliner, J.D., 1999. v *Cell*, 98(5), pp.663-673.
10. Hewitt, S.M., Hamada, S., McDonnell, T.J., Rauscher, F.J. and Saunders, G.F., 1995. Regulation of the proto-oncogen -2 and c-myc by the Wilms' tumor suppressor gene WT1. *Cancer research*, 55(22), pp.5386-5389¹².
11. Wagner, K.D., Wagner, N., Vidal, V.P., Schley, G., Wilhelm, D., Schedl, A., Englert, C. and Scholz, H., 2002. The Wilms' tumor gene Wt1 is required for normal development of the retina. *The EMBO journal*, 21(6), pp.1398-1405.

1
2 12.Silberstein, G.B., Van Horn, K., Strickland, P., Roberts, C.T. and Daniel, C.W., 1997. Altered
3 expression of the WT1 Wilms tumor suppressor gene in human breast cancer. *Proceedings of the*
4 *National Academy of Sciences*, 94(15), pp.8132-8137.
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27

28 **Table 1: WT1 is differentially expressed in the primary tumors of breast cancer patients treated
with trastuzumab.**

Rank	ID	p-value	t	B	FC	Gene
18	GI_13386511-A-4	0.013526	2.56302	-3.41	2.28 ± 2.14	WT1

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 WT1 between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Table 2: WT1 is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

Rank	ID	p-value	t	B	Gene
8485	ILMN_2366967	3.21E-02	2.2296392	-3.99159	WT1

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: WT1 is higher levels in the treated with trastuzumab as compared to those not treated with trastuzumab.

expressed at significantly primary tumors of patients as compared to those not

The mRNA expression level of WT1 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 WT1 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.