The Hedgehog transcription factor GLI3 is differentially expressed in the tumors of breast cancer patients treated with trastuzumab.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Trastuzumab, a monoclonal antibody that targets HER2, is utilized for the treatment of breast cancer¹. While the mechanism of action of trastuzumab function in breast cancer is ascribed to trastuzumab binding to the extracellular region of HER2², the transcriptional consequences that follow this binding event are less well understood. We report here, using analysis of published microarray and gene expression data^{3,4}, that the transcription factor GLI3⁵⁻⁸ is differentially expressed in the tumors of patients with breast cancer treated with trastuzumab.

Keywords: breast cancer, trastuzumab, GLI3, systems biology of breast cancer, targeted therapeutics in breast cancer.

Central nervous system metastases can affect women with breast cancer, but the rate of CNS metastases in breast cancer patients treated with trastuzumab was described at 34% in one report, and said to be higher than one might anticipate in the absence of trastuzumab administration⁹. The mechanism of action ascribed to trastuzumab is thought to be binding of trastuzumab to HER2² but the transcriptional consequences resulting from HER2 binding are less well understood. We found that the Hedgehog transcription factor GLI3, a transcription factor with functions in specification of the patterning and specifications of cell types in the central nervous system⁵⁻⁸ was among the genes most differentially expressed in the primary tumors of patients with breast cancer treated with trastuzumab.

Methods

We used datasets GSE38057³ and GSE130788⁴ 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. 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 GLI3 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 of 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).

GLI3 is differentially expressed in the tumors of patients treated with trastuzumab.

We identified the Hedgehog transcription factor GLI3 as among the genes whose expression changed most significantly in the tumors of breast cancer patients treated with trastuzumab³ (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, GLI3 ranked 27 out of 1506 total transcripts (Table 1), equating to 98.2% differential expression. GLI3 differential expression in the tumors of patients treated with trastuzumab was statistically significant (Table 1; $p=0.017968$).

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 - 41000 genes in total. We found again that GLI3 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, GLI3 ranked 7020 out of 41000 total transcripts (Table 2), equating to 82.9% differential expression. Differential expression of GLI3 was statistically significant (Table 2; $p=7.44E-10$).

1 **GLI3 is expressed at significantly higher levels in the tumors of breast cancer patients**
2 **treated with trastuzumab than in the tumors of those not treated with trastuzumab.**

3 We obtained exact mRNA expression levels for GLI3 from tumors of patients treated with
4 trastuzumab and from the tumors of patients not treated with trastuzumab to directly evaluate magnitude
5 and change of expression in GLI3 based on history of trastuzumab treatment. GLI3 was expressed at
6 significantly higher levels in the tumors of patients treated with trastuzumab as compared to those of
7 patients not treated with trastuzumab (Figure 1; $p=0.0043$). We calculated a mean fold change of $1.83 \pm$
8 1.25 in GLI3 expression in the primary tumors of patients treated with trastuzumab relative to that of
9 patients not treated with trastuzumab.

10 Thus, we found that the Hedgehog transcription factor GLI3 was among the genes most
11 differentially expressed in the tumors of patients treated with trastuzumab, that GLI3 was expressed at
12 significantly higher levels in the tumors of patients treated with trastuzumab as compared to those not
13 treated with trastuzumab.

14 **Discussion**

15 GLI3 has important functions in the development of the hem of the cerebral cortex⁵, in the
16 specification of ventral neurons⁶, in establishing an anterior/posterior gradient in the developing limb⁷,
17 and in the patterning of the mammalian spinal cord through Sonic hedgehog⁸.

18 We provided evidence here that a transcription factor with important developmental functions in
19 the cerebral cortex, ventral neurons and spinal cord is among the genes whose expression changes most
20 significantly in the tumors of patients treated with trastuzumab, a treatment associated with a 34%
21 incidence of central nervous system metastases in patients with breast cancer⁹.

References

1. Herceptin (Trastuzumab). Indications. <https://www.herceptin.com/hcp/indications.html>.
2. Hudis, C.A., 2007. Trastuzumab—mechanism of action and use in clinical practice. *New England Journal of Medicine*, 357(1), pp.39-51.
3. 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.
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. Litingtung, Y. and Chiang, C., 2000. Specification of ventral neuron types is mediated by an antagonistic interaction between Shh and Gli3. *Nature neuroscience*, 3(10), pp.979-985.
6. Grove, E.A., Tole, S., Limon, J., Yip, L.W. and Ragsdale, C.W., 1998. The hem of the embryonic cerebral cortex is defined by the expression of multiple Wnt genes and is compromised in Gli3-deficient mice. *Development*, 125(12), pp.2315-2325.
7. Wang, B., Fallon, J.F. and Beachy, P.A., 2000. Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. *Cell*, 100(4), pp.423-434.
8. Bai, C.B., Stephen, D. and Joyner, A.L., 2004. All mouse ventral spinal cord patterning by hedgehog is Gli dependent and involves an activator function of Gli3. *Developmental cell*, 6(1), pp.103-115.
9. 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
27	GI_13518031-S-2	0.017968	2.44928	-3.53	1.83 ± 1.25	GLI3

Table 1: GLI3 is differentially expressed in the 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 GLI3 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
7020	A_23_P111531	7.44E-10	6.469542	11.429284	GLI3	GLI family zinc finger 3

Table 2: GLI3 is differentially expressed in the 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: GLI3 is expressed at significantly higher levels in the primary tumors of breast cancer patients treated with trastuzumab.

The mRNA expression level of GLI3 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 GLI3 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.