Trastuzumab supports transcription of genes associated with proliferation in human breast cancer.

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The mechanism of action associated with trastuzumab function in breast cancer is thought to involve binding of the F_{ab} domain of trastuzumab to the extracellular portion of the human epidermal growth factor receptor 2 (HER2)¹, but the transcriptional changes induced by signals transduced by this binding event are less described. We mined published and public microarray data, in addition to multiplexed gene expression profiling datasets²⁻⁵ to understand in an unbiased fashion and at the systems level genes most differentially expressed in the tumors of breast cancer patients treated with trastuzumab. We discovered significant perturbation of genes associated with cell proliferation - most significantly but not restricted to the proliferating cell nuclear antigen, PCNA. Significant transcriptional induction of PCNA in the primary tumors of patients with breast cancer following administration of trastuzumab alludes to a seemingly paradoxical role for trastuzumab in support of tumor cell proliferation in human breast cancer.

Keywords: breast cancer, trastuzumab, metastasis, central nervous system metastasis, brain metastasis, systems biology of breast cancer, targeted therapeutics in breast cancer.

Cancer is defined by the inability of a tumor cell to control cell division⁶, resulting in propagation of a population of tumor cells in circulation in blood cancers, or in the generation of a solid tumor in the tissues. Chemotherapeutics and pharmaceuticals utilized in the treatment of cancer can function through a variety of mechanisms; often, cell proliferation is inhibited or impaired following drug treatment, but at times, cancer therapeutics can function in mechanisms independent of cell proliferation. However, propagation of cell proliferation - support of uncontrolled cell division - is antithetical to the very principle by which cancer therapeutics function. In a systems-level transcriptome analysis of primary tumors of the breast from patients treated with trastuzumab, we found markedly enhanced gene expression of the proliferating cell nuclear antigen, PCNA. Further analysis revealed indication of transcriptional induction of the protein kinase AKT1 as well as significantly increased expression of the AP-1 transcription factors FOS and JUNB in the primary tumors of breast cancer patients treated with trastuzumab, each of which are important components of transcriptional programs that support rather than antagonize cell proliferation. These data illuminate seemingly paradoxical transcriptional consequences of a therapeutic with indications for the treatment of a disease characterized by uncontrolled cell proliferation.

Methods

We used datasets GSE38057², GSE114082³, GSE75685⁴, and GSE22226⁵ for this global and multiplexed differential gene expression analysis in conjunction with GEO2R. GSE38057 was generated using DASL Human Cancer Panel by Probe technology with either $n=11$ or $n=17$ primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=36$ or $n=70$ 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. GSE75685 was generated using Whole Human Genome Microarray 4x44K technology with $n=41$ primary breast tumors from patients treated with chemotherapy and $n=11$ primary tumors from patients treated with chemotherapy and trastuzumab, analysis performed using platform GPL4133. 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. 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 PCNA, AKT1, FOS, JUNB gene expression was significantly between primary breast tumors of patients receiving trastuzumab or not 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 mined published and public microarray data as well as multiplexed gene expression profiling datasets²⁻⁵ to perform blind, transcriptome-level analysis of the response to trastuzumab in patients with breast cancer.

PCNA is among the genes whose expression changes most significantly in the tumors of breast cancer patients treated with trastuzumab.

We found that the gene encoding the proliferating nuclear cell antigen, PCNA, 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, PCNA ranked 46 out of 1506 total transcripts, ranking at 96.9% statistical significance (Table 1). Differential expression of PCNA in the primary tumors of breast cancer patients treated with trastuzumab trended towards statistical significance (Table 1; $p=0.065588$).

We validated transcriptome-wide differential expression of PCNA in a separate microarray dataset³ (Table 2). In this second dataset, we compared tumors from the same patients, before and after treatment with trastuzumab. When sorting each of the genes expressed in the primary tumors of patients with breast cancer treated with trastuzumab as compared to those not treated with trastuzumab, PCNA ranked 5114 out of 29377 total transcripts, equating to 82.6% differential expression (Table 2). Differential expression of PCNA transcriptome-wide in the tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=6.87E-03$). Since we compared tumor tissue from the same patients before and after a single treatment with trastuzumab, together we can conclude with confidence that trastuzumab is the cause of, and not simply associated with, induction of PCNA expression.

PCNA is a canonical marker of cell proliferation⁶⁻¹². As such, we continued our analysis to search for any other transcriptional indications of cellular proliferation being modulated by trastuzumab.

Protein kinase AKT1 is among the genes whose expression changes most significantly in the tumors of breast cancer patients treated with trastuzumab.

We found that AKT1, a molecule with key functions in promotion of cell proliferation¹³⁻¹⁷, 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, AKT1 ranked 148 out of 1506 total transcripts (Table 3). Differential expression of AKT1 in the primary tumors of breast cancer patients treated with trastuzumab was not deemed statistically significant (Table 3; $p=0.120178$) despite equating to 90.2% differential expression.

We validated transcriptome-wide differential expression of AKT1 in a separate microarray dataset⁴ (Table 4). When sorting each of the genes expressed in the primary tumors of patients with breast cancer treated with trastuzumab as compared to those not treated with trastuzumab, AKT1 ranked 1255 out of 45220 total transcripts (Table 4), equating to 97.2% differential expression. Differential expression of AKT1 transcriptome-wide in the tumors of patients treated with trastuzumab was statistically significant (Table 4; $p=1.36E-03$).

The AP-1 transcriptional pathway supports mitogen-activated proliferation signals to support cell proliferation. We identified marked transcriptional activation of both FOS and JUNB¹⁸⁻²⁵ in the primary tumors of breast cancer patients treated with trastuzumab.

FOS is among the genes whose expression changes most significantly in the tumors of breast cancer patients treated with trastuzumab.

The AP-1 transcription factor FOS 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 breast cancer patients not treated with trastuzumab, FOS ranked 111 out of 1506 total transcripts (Table 5). Differential expression of FOS in the primary tumors of breast cancer patients treated with trastuzumab was not statistically significant (Table 5; $p=0.084916$).

We validated transcriptome-wide differential expression of FOS in a separate microarray dataset⁵ (Table 6). When sorting each of the genes expressed in the primary tumors of patients with breast cancer treated with trastuzumab as compared to those not treated with trastuzumab, FOS ranked 4562 out of 29377 total transcripts (Table 6). Differential expression of FOS transcriptome-wide in the tumors of breast cancer patients treated with trastuzumab was statistically significant (Table 6; $p=4.82E-03$). JUNB is among the genes whose expression changes most significantly in the tumors of breast cancer patients treated with trastuzumab.

Another AP-1 transcription factor, JUNB was also among the genes whose expression was most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab. 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, JUNB ranked 162 out of 1506 total transcripts (Table 7). Differential expression of JUNB in the primary tumors of breast cancer patients treated with trastuzumab was not statistically significant (Table 7; $p=0.127726$), but equated to 89.2% differential expression.

We validated transcriptome-wide differential expression of JUNB in a separate microarray dataset⁶ (Table 8). When sorting each of the genes expressed in the primary tumors of patients with breast cancer treated with trastuzumab as compared to those not treated with trastuzumab, JUNB ranked 5972 out of 44290 total transcripts (Table 8). Differential expression of JUNB transcriptome-wide in the primary tumors of breast cancer patients treated with trastuzumab was not deemed statistically significant (Table 8; $p=9.21E-02$), but equated to 86.5% differential expression.

Thus, after identifying that PCNA, AKT1, FOS and JUNB were all among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab, we sought to discern whether these genes were increasing or decreasing in value following administration of trastuzumab.

PCNA is transcriptionally induced in the tumors of breast cancer patients treated with trastuzumab.

We obtained exact mRNA expression levels for PCNA in the primary tumors of patients treated with trastuzumab and those not treated with trastuzumab to evaluate the magnitude and direction of PCNA modulation in the tumors of patients treated with trastuzumab. Surprisingly, we found that PCNA expression increased rather than decreased in patients treated with trastuzumab (Figure 1). Moreover, this increase was statistically significant (Figure 1; $p=0.0015$). We calculated a 1.34 ± 0.68 mean fold change of PCNA in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab (Table 1).

AKT1 is transcriptionally induced in the tumors of breast cancer patients treated with trastuzumab.

We also obtained exact mRNA expression levels for AKT1 in the primary tumors of patients treated with trastuzumab and those not treated with trastuzumab to evaluate the magnitude and direction of AKT1 modulation in the tumors of patients treated with trastuzumab. We found that that like PCNA, AKT1 expression increased rather than decreased in patients treated with trastuzumab (Figure 2).

This increase approached the level of statistical significance (Figure 2; $p=0.0768$). We calculated a 1.20 ± 0.39 mean fold change of AKT1 in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab (Table 2).

FOS is expressed at higher levels in the tumors of breast cancer patients treated with trastuzumab.

Next we obtained exact mRNA expression levels for FOS in the primary tumors of patients treated with trastuzumab and those not treated with trastuzumab to evaluate the magnitude and direction of FOS modulation in the tumors of patients treated with trastuzumab. We found that that like PCNA, AKT1 and JUNB, FOS expression increased rather than decreased in patients treated with trastuzumab (Figure 3). Again, this increase was statistically significant (Figure 3; $p=0.0033$). We calculated a 1.23 ± 0.39 mean fold change of FOS in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab (Table 3).

JUNB is expressed at higher levels in the tumors of breast cancer patients treated with trastuzumab.

Finally, we obtained exact mRNA expression levels for JUNB in the primary tumors of patients treated with trastuzumab and those not treated with trastuzumab to evaluate the magnitude and direction of JUNB modulation in the tumors of patients treated with trastuzumab. We found that that like PCNA and AKT1, JUNB expression increased rather than decreased in patients treated with trastuzumab (Figure 4). Again, this increase was statistically significant (Figure 4; $p=0.0498$). We calculated a 1.34 ± 0.64 mean fold change of JUNB in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab (Table 4).

Thus, using published microarray and multiplexed gene expression profiling data to perform blinded, systems-level transcriptome analysis of primary tumor tissue from human patients with breast cancer, we found that that trastuzumab treatment in breast cancer is associated with transcriptional induction of PCNA, and significantly increased expression of AKT1, FOS and JUNB. Transcriptional induction of PCNA and increased expression of AKT1, FOS and JUNB are not simply marginal artifacts of trastuzumab treatment arrived at through contrived hypothesis testing; PCNA, AKT1, FOS and JUNB are among the genes whose expression changes most significantly transcriptome-wide when comparing the primary tumors of breast cancer patients treated with trastuzumab as compared to patients not treated with trastuzumab.

Discussion

Despite the fact that cancer is defined by uncontrolled cell proliferation, we found here that administration of trastuzumab to patients with breast cancer is associated with a marked and significant induction of PCNA, a marker of cell proliferation, transcriptional activation of trans-acting factors regulating cell growth gene expression programs - the AP-1 transcription factors FOS and JUNB - and a protein kinase with critical roles in cell proliferation, AKT1.

Future studies can evaluate whether activation or suppression of other genes can mitigate the real risk of inducing cellular proliferation in patients with active tumor growth, or whether induction of multiple genes associated with cell proliferation, in normal cellular biology and in tumor cells, is an overlooked byproduct of treatment with an antibody possessing both an F_{ab} and F_c domain that could function independently and potentially cooperatively to transduce signals that promote rather than restrict tumor growth in patients with breast cancer.

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. GSE75685. Mexican Patients with Breast Cancer. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE75685>. Hugo Gomez, Bioinformatics, ITESM, Mexico.
5. 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.
6. Evan, G.I. and Vousden, K.H., 2001. Proliferation, cell cycle and apoptosis in cancer. *Nature*, 411(6835), pp.342-348.
7. Dietrich, D.R., 1993. Toxicological and pathological applications of proliferating cell nuclear antigen (PCNA), a novel endogenous marker for cell proliferation. *Critical reviews in toxicology*, 23(1), pp. 77-109.
8. Kubben, F.J., Peeters-Haesevoets, A., Engels, L.G., Baeten, C.G., Schutte, B., Arends, J.W., Stockbrügger, R.W. and Blijham, G.H., 1994. Proliferating cell nuclear antigen (PCNA): a new marker to study human colonic cell proliferation. *Gut*, 35(4), pp.530-535.
9. Connolly, K.M. and Bogdanffy, M.S., 1993. Evaluation of proliferating cell nuclear antigen (PCNA) as an endogenous marker of cell proliferation in rat liver: a dual-stain comparison with 5-bromo-2'-deoxyuridine. *Journal of Histochemistry & Cytochemistry*, 41(1), pp.1-6.
10. Iatropoulos, M.J. and Williams, G.M., 1996. Proliferation markers. *Experimental and Toxicologic Pathology*, 48(2-3), pp.175-181.
11. Foley, J.F., Dietrich, D.R., Swenberg, J.A. and Maronpot, R.R., 1991. Detection and evaluation of proliferating cell nuclear antigen (PCNA) in rat tissue by an improved immunohistochemical procedure. *Journal of Histochemistry*, 14(4), pp.237-241.
12. Robbins, B.A., De la Vega, D., Ogata, K., Tan, E.M. and Nakamura, R.M., 1987. Immunohistochemical detection of proliferating cell nuclear antigen in solid human malignancies. *Archives of pathology & laboratory medicine*, 111(9), pp.841-845.

13. Héron-Milhavet, L., Franckhauser, C., Rana, V., Berthenet, C., Fisher, D., Hemmings, B.A., Fernandez, A. and Lamb, N.J., 2006. Only Akt1 is required for proliferation, while Akt2 promotes cell cycle exit through p21 binding. *Molecular and cellular biology*, 26(22), pp.8267-8280.
14. Liu, H., Radisky, D.C., Nelson, C.M., Zhang, H., Fata, J.E., Roth, R.A. and Bissell, M.J., 2006. Mechanism of Akt1 inhibition of breast cancer cell invasion reveals a protumorigenic role for TSC2. *Proceedings of the National Academy of Sciences*, 103(11), pp.4134-4139.
15. Moriya, N. and Miyazaki, M., 2018. Akt1 deficiency diminishes skeletal muscle hypertrophy by reducing satellite cell proliferation. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 314(5), pp.R741-R751.
16. Chang, Z., Zhang, Q., Feng, Q., Xu, J., Teng, T., Luan, Q., Shan, C., Hu, Y., Hemmings, B.A., Gao, X. and Yang, Z., 2010. Deletion of Akt1 causes heart defects and abnormal cardiomyocyte proliferation. *Developmental biology*, 347(2), pp.384-391.
17. Ju, X., Katiyar, S., Wang, C., Liu, M., Jiao, X., Li, S., Zhou, J., Turner, J., Lisanti, M.P., Russell, R.G. and Mueller, S.C., 2007. Akt1 governs breast cancer progression in vivo. *Proceedings of the National Academy of Sciences*, 104(18), pp.7438-7443.
18. Angel, P. and Karin, M., 1991. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, 1072(2-3), pp.129-157.
19. Shaulian, E. and Karin, M., 2001. AP-1 in cell proliferation and survival. *Oncogene*, 20(19), pp.2390-2400.
20. van der Burg, B., van Selm-Miltenburg, A.J., de Laat, S.W. and van Zoelen, E.J., 1989. Direct effects of estrogen on c-fos and c-myc protooncogene expression and cellular proliferation in human breast cancer cells. *Molecular and cellular endocrinology*, 64(2), pp.223-228.
21. Dahlman-Wright, K., Qiao, Y., Jonsson, P., Gustafsson, J.Å., Williams, C. and Zhao, C., 2012. Interplay between AP-1 and estrogen receptor α in regulating gene expression and proliferation networks in breast cancer cells. *Carcinogenesis*, 33(9), pp.1684-1691.
22. Passequé, E., Jochum, W., Behrens, A., Ricci, R. and Wagner, E.F., 2002. JunB can substitute for Jun in mouse development and cell proliferation. *Nature genetics*, 30(2), pp.158-166.
23. Mathas, S., Hinz, M., Anagnostopoulos, I., Krappmann, D., Lietz, A., Jundt, F., Bommert, K., Mehta-Grigoriou, F., Stein, H., Dörken, B. and Scheidereit, C., 2002. Aberrantly expressed c-Jun and JunB are a hallmark of Hodgkin lymphoma cells, stimulate proliferation and synergize with NF- κ B. *The EMBO journal*, 21(15), pp.4104-4113.
24. Jia, J., Ye, T., Cui, P., Hua, Q., Zeng, H. and Zhao, D., 2016. AP-1 transcription factor mediates VEGF-induced endothelial cell migration and proliferation. *Microvascular research*, 105, pp.103-108.
25. Fan, F., Bashari, M.H., Morelli, E., Tonon, G., Malvestiti, S., Vallet, S., Jarahian, M., Seckinger, A., Hose, D., Bakiri, L. and Sun, C., 2017. The AP-1 transcription factor JunB is essential for multiple myeloma cell proliferation and drug resistance in the bone marrow microenvironment. *Leukemia*, 31(7), pp.1570-1581.

Rank	ID	p-value	t	B	FC	Gene
46	GI_33239450-A-1	0.065588	1.864266	-4.2	1.34 ± 0.68	PCNA

Table 1: PCNA 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, the fold change (FC) of PCNA mRNA expression between the tumors of the patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	Gene
5114	ILMN_1694177	6.87E-03	-2.8686074	-2.63193	PCNA

Table 2: PCNA 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, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene
148	GI_4885060-S-4	0.120178	1.58182	-4.33	1.20 ± 0.39	AKT1

Table 3: AKT1 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, the fold change (FC) of AKT1 mRNA expression between the tumors of the patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
1255	4669	1.36E-03	3.3851421	-1.088376	2.113746	AKT1	AKT serine/ threonine kinase 1

Table 4: AKT1 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, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene
111	GI_6552332-S-5	0.084916	1.75875	-4.19	1.23 ± 0.39	FOS

Table 5: FOS 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, the fold change (FC) of FOS mRNA expression between the tumors of the patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	Gene
4562	ILMN_1669523	4.82E-03	3.0054923	-2.31239	FOS

Table 6: FOS 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, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene
162	GI_4504808-S-4	0.127726	1.5496	-4.35	1.34 ± 0.64	JUNB

Table 7: JUNB 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, the fold change (FC) of JUNB mRNA expression between the tumors of the patients treated with trastuzumab as compared to the tumors of patients not treated with trastuzumab, and gene symbol are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
5972	37209	9.21E-02	-1.70	-4.39315	JUNB	JunB proto-oncogene, AP-1 transcription factor subunit

Table 8: JUNB 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, and gene symbol are listed in this chart.

Figure 1: PCNA is expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of PCNA is graphically depicted in the tumors of patients not treated with trastuzumab (left) and in the tumors of patients treated with trastuzumab (right), with mean mRNA expression levels depicted and the result of a statistical test evaluating significance of difference in PCNA mRNA expression level between patients not treated with trastuzumab and patients with trastuzumab, a *p*-value, listed above.

Figure 2: AKT1 is expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of AKT1 is graphically depicted in the tumors of patients not treated with trastuzumab (left) and in the tumors of patients treated with trastuzumab (right), with mean mRNA expression levels depicted and the result of a statistical test evaluating significance of difference in AKT1 mRNA expression level between patients not treated with trastuzumab and patients with trastuzumab, a *p*-value, listed above.

Figure 3: FOS is expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of FOS is graphically depicted in the tumors of patients not treated with trastuzumab (left) and in the tumors of patients treated with trastuzumab (right), with mean mRNA expression levels depicted and the result of a statistical test evaluating significance of difference in FOS mRNA expression level between patients not treated with trastuzumab and patients with trastuzumab, a *p*-value, listed above.

Figure 4: JUNB is expressed at significantly higher levels in the tumors of patients treated with trastuzumab as compared to those not treated with trastuzumab.

The mRNA expression level of JUNB is graphically depicted in the tumors of patients not treated with trastuzumab (left) and in the tumors of patients treated with trastuzumab (right), with mean mRNA expression levels depicted and the result of a statistical test evaluating significance of difference in JUNB mRNA expression level between patients not treated with trastuzumab and patients with trastuzumab, a *p*-value, listed above.