Bcl-XL (BCL2L1) is differentially expressed in the primary tumors of breast cancer patients treated with trastuzumab.

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Trastuzumab, a monoclonal antibody targeted against the human epidermal growth factor receptor 2 (HER2) is utilized for the treatment of human breast cancer^{1,2}, but a complete understanding of how tumor signal transduction is modulated by trastuzumab treatment is lacking. By mining published and public microarray and gene expression data^{3,4} from the primary tumors of patients treated with trastuzumab, we found that anti-apoptotic protein Bcl-XL, also known as BCL2L1 was among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab. BCL2L1 messenger RNA expression was markedly enhanced in the primary tumors of patients treated with trastuzumab. Thus, trastuzumab treatment in patients with breast cancer is associated with increased expression, in primary tumors of the breast, of cellular machinery that prevents cell death⁵⁻⁹.

Keywords: breast cancer, trastuzumab, BCL2L1, systems biology of breast cancer, targeted therapeutics in breast cancer.

1 The mechanism of action by which trastuzumab exerts its therapeutic function in human breast
2 cancer is said to be binding of the F_{ab} domain of trastuzumab to the extracellular region of the human
3 epidermal growth factor receptor 2 (HER2)², possibly resulting in decreased cellular proliferation⁸.
4 Transcriptional modulation resulting from trastuzumab binding events in the primary tumors of treated
5 patients is less well understood. We mined published and public microarray and gene expression data^{3,4}
6 generated using primary tissues from patients treated with trastuzumab to compare tumor global gene
7 expression with that of patients not treated with trastuzumab. Using this unbiased, systems level
8 methodology, we identified differential expression and up-regulation of BCL2L1 in primary tumors of the
9 breast in patients treated with trastuzumab.

10 **Methods**

11 We used datasets GSE38057³ and GSE130788⁴ for this differential gene expression analysis of
12 the transcriptional response to trastuzumab treatment in patients with breast cancer. GSE38057 was
13 generated using DASL Human Cancer Panel by Probe technology with $n=6$ primary breast tumors from
14 patients treated with induction chemotherapy and/or hormone therapy without trastuzumab and $n=35$
15 primary breast tumors from patients treated with induction chemotherapy and/or hormone therapy in
16 addition to trastuzumab, both of which developed brain metastases; analysis was performed using
17 platform GPL5826. GSE130788 was generated using Agilent-014850 Whole Human Genome
18 Microarray 4x44K G4112F technology with $n=11$ primary breast tumors from patients treated with
19 trastuzumab and $n=36$ primary tumors from patients treated with trastuzumab, analysis performed using
20 platform GPL6480. The Benjamini and Hochberg method of p -value adjustment was used for ranking of
21 differential expression but raw p -values were used to assess statistical significance of global or
22 multiplexed differential expression. Log-transformation of data was auto-detected, and the NCBI
23 generated category of platform annotation was used. A statistical test was performed to evaluate whether
24 BCL2L1 gene expression was significantly between primary breast tumors of patients receiving
25 trastuzumab using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical
26 analyses of differential gene expression in human breast cancer (Version 8.4.0)(455).

27 **Results**

28 We performed comparative transcriptome analysis of primary tumors from patients treated or not
with trastuzumab (Herceptin), using published multiplexed gene expression data³ and a microarray
dataset⁴, in order to identify the most significant transcriptional changes in the primary tumors of breast
cancer patients following treatment with trastuzumab.

BCL2L1 is differentially expressed in the primary tumors of patients treated with trastuzumab.

We found that BCL2L1, also known as Bcl-XL, was among the genes whose expression was
most significantly different in the primary tumors of patients with breast cancer treated with trastuzumab³.
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, BCL2L1 ranked 6 out of 1506 total transcripts, equating to 99.6% statistical significance
(Table 1). Differential expression of BCL2L1 in the primary tumors of breast cancer patients treated with
trastuzumab was trended towards statistical significance (Table 1; $p=0.00882$).

We queried a second dataset⁶ to validate our findings of BCL2L1 differential expression upon
trastuzumab treatment. In a second microarray dataset analyzing gene expression of patients treated with
trastuzumab, we again found that BCL2L1 was among the genes whose expression was most significantly

different transcriptome-wide when comparing the global gene expression profiles of 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 patients treated with trastuzumab based on significance of change as compared to the tumors of patients not treated with trastuzumab, BCL2L1 ranked 806 out of 41000 total transcripts, equating to 98.0% differential expression. Differential expression of BCL2L1 in the primary tumors of patients treated with trastuzumab was statistically significant (Table 2; $p=6.85E-29$).

BCL2L1 is expressed at higher levels in the tumors of patients treated with trastuzumab.

We obtained exact mRNA expression levels for BCL2L1 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of BCL2L1 change in the tumors of patients treated with trastuzumab. BCL2L1 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 BCL2L1 trended towards statistical significance (Figure 1: $p=0.0974$). We calculated a mean fold change of 1.57 ± 0.75 when comparing the expression of BCL2L1 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that the anti-apoptotic molecule BCL2L1 (Bcl-XL) was among the genes most differentially expressed in the primary tumors of patients with breast cancer and that BCL2L1 mRNA was present at higher quantities in the primary tumors of patients treated with trastuzumab as compared to those not treated.

Discussion

We provided evidence here that BCL2L1, also known as Bcl-XL, is expressed at higher levels in the tumors of patients treated with trastuzumab and among the genes most differentially expressed in the primary tumors of patients treated with trastuzumab. Thus, trastuzumab administration in patients with breast cancer is associated with enhanced primary tumor expression of an anti-apoptotic molecule⁵⁻⁹, therefore linking trastuzumab with cell survival pathways rather than cell death.

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Rank	ID	p-value	t	B	FC	Gene
6	GI_20336333-A-3	0.00882	2.744161	-4.11	1.57 ± 0.75	BCL2L1

Table 1: BCL2L1 (Bcl-XL) 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 BCL2L1 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
806	A_23_P309996	6.85E-29	-13.163481	54.845647	BCL2L11	BCL2 like 11

Table 2: BCL2L1 (Bcl-XL) 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 BCL2L1 between the tumors of patients treated with trastuzumab and the tumors of patients not treated with trastuzumab, and gene are listed in this chart.

Figure 1: BCL2L1 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 BCL2L1 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 BCL2L1 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.