The LMO2 oncogene is up-regulated in the primary tumors of patients treated with trastuzumab.

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We report here that in human breast cancer, trastuzumab treatment is associated with significantly increased expression of the oncogene LMO2¹⁻³. This piece of evidence, obtained through unbiased comparative transcriptome analysis of published data^{4,5}, in conjunction with published data demonstrating roles for LMO2 in transformation of T-cells and in specifying tumorigenic properties of glioma stem cells¹⁻³, continues to illustrate a portrait of a cancer therapeutic with molecular properties associated with proliferation and metastasis, helping to reconcile a 34% incidence of central nervous system metastases⁶ in patients treated with what is considered a front-line treatment for adjuvant and metastatic breast cancer.

Keywords: breast cancer, cell cycle, LMO2, systems biology of breast cancer, targeted therapeutics in breast cancer.

1 The mechanism of action ascribed to trastuzumab (Herceptin) function is thought to be binding of
2 the Fab domain of trastuzumab to the extracellular region of the human epidermal growth factor receptor
3 2 (HER2)⁷, resulting in impairment of cellular proliferation, signal transduction downstream of this
4 binding event and transcriptional consequences of this signaling is less well understood. We used
5 systems-level, unbiased transcriptome analysis of published data^{4,5} to discover genes transcriptionally
6 perturbed in primary tumors of patients treated with trastuzumab, a monoclonal antibody targeting HER2,
7 finding not only that the oncogene LMO2 was among the genes most differentially expressed in the
8 tumors of patients treated with trastuzumab, but that it was expressed at significantly higher levels than in
9 patients not treated with trastuzumab.

10 **Methods**

11 We used datasets GSE38057⁴ and GSE75685⁵ for this differential gene expression analysis of the
12 transcriptional landscape of trastuzumab. GSE38057 was generated using DASL Human Cancer Panel by
13 Probe technology with either $n=11$ primary breast tumors from patients treated with induction
14 chemotherapy and/or hormone therapy without trastuzumab and $n=36$ primary breast tumors from
15 patients treated with induction chemotherapy and/or hormone therapy in addition to trastuzumab; analysis
16 was performed using platform GPL5826. GSE75685 was generated using Whole Human Genome
17 Microarray 4x44K technology with $n=41$ primary breast tumors from patients not treated with
18 trastuzumab and $n=11$ primary tumors from patients treated with trastuzumab, analysis performed using
19 platform GPL4133. The Benjamini and Hochberg method of p -value adjustment was used for ranking of
20 differential expression but raw p -values were used to assess statistical significance of global or
21 multiplexed differential expression. Log-transformation of data was auto-detected, and the NCBI
22 generated category of platform annotation was used. A statistical test was performed to evaluate whether
23 LMO2 gene expression was significantly between primary breast tumors of patients receiving
24 trastuzumab using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical
25 analyses of differential gene expression in human breast cancer (Version 8.4.0)(455).

26 **Results**

27 We performed systems-level comparative transcriptome analysis to describe in an unbiased
28 fashion the most significant transcriptional features of trastuzumab treatment in patients with breast
cancer, using published microarray and multiplexed gene expression data^{4,5}.

29 **LMO2 is differentially expressed in the primary tumors of patients treated with trastuzumab.**

30 We found that the gene encoding the oncogene LMO2 was among the genes whose expression
31 was most significantly different in the primary tumors of patients with breast cancer treated with
32 trastuzumab⁴. When sorting each of the genes expressed in trastuzumab-treated breast tumors based on
33 significance of difference in expression as compared to the primary breast tumors of
34 patients not treated with trastuzumab, LMO2 ranked 65 out of 1506 total transcripts, ranking at 95.7%
35 statistical significance (Table 1). Differential expression of LMO2 in the primary tumors of breast cancer
36 patients treated with trastuzumab was statistically significant (Table 1; $p=0.053006$).

37 We sought to validate our findings of LMO2 differential expression upon trastuzumab treatment
38 in an independent dataset. In a second microarray dataset analyzing gene expression of patients treated
with trastuzumab⁵, we again found that LMO2 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, LMO2 ranked 8704 out of 45220 total transcripts, equating to 80.8% differential expression. Differential expression of LMO2 was statistically significant (Table 2; $p=5.1E-02$).

LMO2 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 LMO2 from the tumors of patients treated with trastuzumab and from the tumors of patients not receiving trastuzumab to determine magnitude and direction of LMO2 change in the tumors of patients treated with trastuzumab. We were alarmed to find that LMO2 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 LMO2 was statistically significant (Figure 1: $p=0.0106$). We calculated a mean fold change of 1.34 ± 0.43 when comparing the expression of LMO2 in the tumors of patients treated with trastuzumab as compared to the tumors of patients treated with trastuzumab.

Thus, we found that LMO2 was among the most significant transcriptional features of the primary tumors of breast cancer patients treated with trastuzumab, and that LMO2 was expressed at significantly increased levels in the primary tumors of patients treated with trastuzumab.

Discussion

We provided evidence here that messenger RNA for the oncogene LMO2 is present in significantly higher quantities in the tumors of patients treated with trastuzumab than in the tumors of patients never treated with trastuzumab. LMO2 is a potent oncogene whose transgenic expression results in T-cell acute lymphoblastic leukemia in mice^{1,10}, and whose genomic disruption by vector integration during gene therapy has resulted in cancer in humans^{3,8}. LMO2 has also been described to support metastasis in basal-type breast cancer¹². We argue that the increased LMO2 expression associated with trastuzumab treatment we have demonstrated here is a particularly dangerous result of administering trastuzumab to patients with breast cancer.

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Rank	ID	p-value	t	B	FC	Gene
65	GI_6633806-S-5	0.053006	1.98324	-3.99	1.34 ± 0.43	LMO2

Table 1: Differential expression of LMO2 in primary tumors of trastuzumab-treated 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, fold change of expression in LMO2 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
8704	39175	5.1E-02	-1.9975805	-4.197568	LMO2	LIM domain only 2

Table 2: Differential expression of LMO2 in primary tumors of trastuzumab-treated 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: LMO2 is expressed at significantly higher levels in the tumors of breast cancer patients treated with trastuzumab.

The mRNA expression level of LMO2 is graphically depicted in this chart in the primary tumors of breast cancer patients not treated with trastuzumab and in the primary tumors of breast cancer patients treated with trastuzumab, with mean mRNA expression level marked and the result of a statistical test evaluating significance of difference between LMO2 expression in the primary tumors of patients treated with trastuzumab or not, a *p*-value, listed above.