

## Therapeutic targeting in HER2+ breast cancer to prevent and treat CNS disease: DTL.

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Up to 50% of patients with HER2+ subtype breast cancer develop metastasis to the central nervous system, most commonly the brain, demanding rapid therapeutic approaches to limit spread of the HER2+ primary tumor to distant sites (1-3). We recently described the existence of a group of genes that reside proximal to *ERBB2* (the gene that encodes the human epidermal growth factor *HER2*) at 17q12: their differential expression in HER2+ breast cancer, their up-regulation in HER2+ breast cancer, their differential expression and up-regulation in central nervous system (CNS) metastasis and, based on human survival studies, their function in supporting metastasis to the CNS, indicating that the predilection of HER2+ patients to develop CNS metastasis was a phenomena attributable to the disease and not HER2+-targeted therapies (4). Disease recurrence following disease remission (relapse), resistance to trastuzumab or otherwise inadequate long-term control of disease are challenges that limit effectiveness of existing HER2+-targeted therapies. We utilized whole transcriptome technologies (5, 6) to measure total transcription in the primary tumors of humans with HER2+ breast cancer, identifying genes in the HER2 signature based on difference from the luminal A and luminal B tumor transcriptomes. We describe here a candidate therapeutic target up-regulated and differentially expressed in human HER2+ breast cancer, DTL, as a candidate therapeutic target for the prevention and management of CNS metastasis in HER2+ breast cancer.

CRISPR-based reverse genetic screening of human HER2+ organoids or primary tumor cells freshly isolated from patients with HER2+ breast cancer with limited exposure to artificial culture conditions have not yet been described (7, 8). These genetic technologies, though not yet implemented, when done so will likely facilitate discovery of therapeutic vulnerabilities in HER2+ disease with rapidity and ease. Management of major public health problems requires research (bench)-based management in the short run and long run (9). One example of such a major problem is CNS metastasis in HER2+ breast cancer (10-13): this is a type of cancer that affects a human sex with relatively high frequency, a subtype of that cancer that is diagnosed in approximately one quarter of patients diagnosed with that cancer, and a specific complication of that subtype develops in half of affected patients.

We utilize genomic and transcriptomic technologies to study the genomic sequences (DNA), the transcriptome (RNA), and epigenetic modification (eg., CpG-DNA) of humans with breast cancer. This includes the primary tumor, the source of the transformation - like mutant variants of p53 - subtypes of the primary tumor, including luminal, basal and HER2+ forms, "regional" metastasis to the lymph nodes, metastasis to distant sites, including the lungs, the liver and the brain, and the circulating tumor stem cell. Our hypothesis and working strategy dictates that in the short-run, disease complications and therapeutic limitations are best managed using identification of therapeutic targets by whole transcriptome differential expression analyses (subtraction analysis), and in the long-run, will be enhanced and fully complemented by reverse genetic screening strategies to blindly identify disease-specific, subtype-specific and metastasis-specific therapeutic vulnerabilities augmented by novel immunotherapy approaches. Here we describe one such target identified through rigorous study of the HER2+ tumor transcriptome: a therapeutic target that is up-regulated, catalytically available and subtype-specific: DTL.

## Results

**Figure 1:** DTL is differentially expressed in HER2+ breast cancer.

### I. Primary tumors of the breast from humans with breast cancer: HER2+ subtype

*n*=59 primary tumors (breast; human; luminal A and luminal B)

*n*=30 primary tumors (breast; human; HER2+)

ID	<i>p</i> -value	t	B	logFC	Gene	Rank	%DE
222680_s_at	4.17E-05	-4.310553	1.717066	-1.4707	DTL	2837/29873	90.5

Through quantitative comparison of total transcription in luminal subtype primary tumors and in primary tumors of humans with HER2+ breast cancer (5), we discovered differential expression of denticleless E3 ubiquitin protein ligase homolog, encoded by *DTL* in HER2+ breast cancer in humans (**Chart 1**). The expression of DTL changed more than 90% of the human breast tumor transcriptome when considering all transcripts whose expression was measured - in this case, 29,873 transcripts ("Rank"). Note the negative fold-change indicating increased quantity of DTL messenger RNA in HER2+ subtype tumors, demonstrating up-regulation of DTL during transformation and lineage specification of the breast to the HER2+ breast cancer subtype.

### II. Primary tumors of the breast from humans with breast cancer: HER2+ subtype

*n*=77 primary tumors (breast; human; luminal A and luminal B)

*n*=18 primary tumors (breast; human; HER2+)

ID	<i>p</i> -value	t	B	logFC	Gene	Rank	%DE
7909568	2.06E-02	-2.3531033	-3.64267	-0.59620289	DTL	4170/33297	87.5

Through quantitative comparison of total transcription in the primary tumors of humans with HER2+ breast cancers relative to luminal subtype breast cancers, in a second microarray dataset (6) from independent investigators and a separate patient cohort, we validated differential expression of DTL in HER2+ breast cancer in humans (**Chart 2**). The expression of DTL here changed more than 85% of the human breast tumor transcriptome when considering all transcripts whose expression was measured - in this case, 33,297 transcripts ("Rank"). Note the negative fold-change indicating increased quantity of DTL messenger RNA in HER2+ subtype tumors, demonstrating up-regulation of DTL during transformation and lineage specification of the breast to the HER2+ breast cancer subtype.

Thus, differential and increased expression of DTL defines the transcriptional landscape of the HER2+ breast cancer subtype in humans.

## Discussion

Adjunctive treatments in medical oncology limit the emergence of resistant tumor clones during treatment with a second treatment (whether neoadjuvant chemotherapy or a targeted therapy like trastuzumab). Small molecule inhibitors of DTL, once evaluated for toxicity and safety, can immediately be tested for efficacy in patients with HER2+ metastasis, with the goal of identifying the most effective medicines for management of HER2+ disease in humans and utilizing these adjunctive inhibitors early in disease to prevent rather than treat CNS disease. We recently used primary tumor transcriptome data to identify multiple phosphatases that we deemed candidate therapeutic targets in management of breast cancer, and fortuitously found their decreased expression was specifically linked to superior overall survival in patients with HER2+ breast cancer (14, 15). A multi-kinase approach delivered in conjunction with chemotherapies that target dNTP synthesis, replication of the daughter strand and activity at the spindle at anaphase is most likely to be most effective in limiting tumor clone resistance (16).

A description of basic chemotherapy approaches supported by our discovery research follows.

1. **Intensification of standard chemotherapy with MDR pump inhibition** at baseline and at resistance

- a. The pump is the only entity considered at resistance for compensatory clinical inhibition.

2. **Multi-drug** approach targeting DNA replication and cell division: **EZH2, TK1, TYMS, AURKA, and TOP2A**

- a. Adjunctive option 1: Targeted kinase adjunctive

- b. Adjunctive option 2: Targeted phosphatase adjunctive

3. **A general solid tumor dual inhibitor** strategy: PARP1/2 inhibition together with CDK4/6 inhibition

4. Genomic medicine-based (tailored) **combinatorial phosphatase inhibition**.

- a. The number of phosphatases targeted in this approach will depend on tolerability and deliverability of successful targeting combinations.

5. Genomic medicine-based (tailored) **combinatorial kinase inhibition**.

- a. The number of kinases targeted in this approach will depend on tolerability and deliverability of successful targeting combinations

6. **A complete growth factor inhibition** strategy.

- a. Targeting angiogenesis through VEGF-A lies at the basis of this fifth chemotherapeutic strategy.

- b. The approach relies on **accurate and complete description of growth factors induced at the 80-99% range** of the transcriptome in order to completely restrict growth factor signaling

- c. **A reduced cytotoxic regimen** is ideally administered with the growth factor inhibitor cocktail to induce cell death whilst activating starvation responses
- d. **Kinase targeting for compensatory inhibition.**

#### 7. Immunoglobulin-based therapeutic targeting: drug discovery approach.

- a. This approach is agnostic to considerations or concepts that prevail in modern medical oncology like cytotoxicity associated with standard chemotherapies like nitrogen mustard based agents, as well as to considerations common when targeting general properties of the cancer cell, like those considered by the multi-drug approach which aims to block DNA replication and cell division
- b. Instead, it utilizes intensive study of tumor transcriptomes for identification of disease-specific therapeutic vulnerabilities for clinical intervention using targeted, designed monoclonal antibody reagents.
- c. Targets identified here, rather than being assembled into an immunoglobulin-based therapeutic strategy, can be used as an individual agent as adjunctive treatments in other chemotherapeutic approaches.

#### 8. Treatments that target the immune system for control of disease (cancer).

- a. Class I and II antigen presentation by the tumor through **UBE2L6** modulation
- b. Natural killer cell tumor surveillance through **NKG7 and HLA-V together with KIR2DL3**
  - i. Gamma delta T cells may function with NK cells in this pathway.
- c. **Targeting exhaustion** ("reinvigoration") at the tumor microenvironment using pharmacologic inhibition of **CTLA4 AND PD-1**, like involving cytotoxic immune responses by CD8 T lymphocytes
  - i. Novel immunoreceptor targeting TIGIT, LAG-3, VISTA and other surface receptors

#### 9. Rational responses to resistance.

- a. **CDKN re-activation** through CDK4/6 inhibitor escalation
- b. CDKN re-activation through **UBE2C and UBE2S** modulation

#### 10. Targeting **centers of activity** in the cancer cell.

- a. **Kinetochores**
  - i. SPC24/SPC25, Nuf2, NDC80
  - ii. Other components of the kinetochore plate
- b. **Cell membrane** during cytokinesis
  - i. PKC isoforms; ECT2.

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## Methods

We utilized GSE45827 for this tumor transcriptome study, measuring whole transcription in HER2+ primary tumors from humans with breast cancer, as compared to luminal subtype primary tumors (along with GSE87049 for target validation) using microarray data (published) and R-based computational methods (GEO2R).