Pretreatment serum metabolome predicts PFS in first-line trastuzumab-treated metastatic breast cancer.

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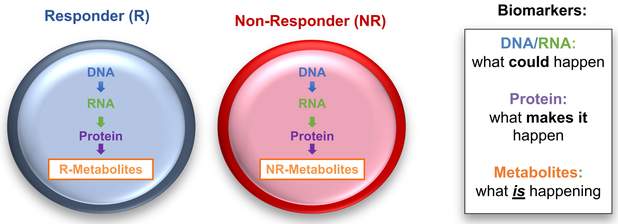
WHAT IS THE PROBLEM?

HER2-positive (HER2+) metastatic breast cancer (mBC) patients have significant heterogeneity in response and progression-free survival (PFS) to Her2-targeted therapy trastuzumab. Several resistance mechanisms have been identified including impaired drug binding to Her2 through receptor variants or molecular masking, constitutive activation of signaling pathways parallel or downstream of Her2 such as CDK4/6-CyclinD, PI3K, AKT, and mTOR pathways, or reduced immune system activation such as escape from antibody-dependent cellular cytotoxicity (ADCC). Overexpression of Fatty Acid Synthase gene (FASN) has also been associated with poor clinical response to anti-Her2 therapy. Few if any biomarkers have been clinically validated to identify patients who will have a durable response to anti-HER2 therapy.

OBJECTIVE(S)

1. To evaluate pretreatment serum metabolite biomarkers for correlation with PFS in a cohort of trastuzumab-treated metastatic breast cancer patients from a single institution.
2. To identify potential metabolic pathways that are altered in patients with limited response to trastuzumab.

WHY METABOLITES?



Biomarkers:

DNA/RNA: what **could** happen

Protein: what **makes it** happen

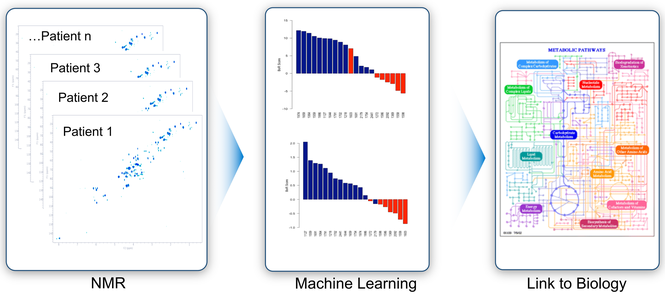
Metabolites: what ***is*** happening

Cancer cells have altered metabolism, which contributes to their ability to proliferate, survive in unusual microenvironments, and invade other tissues. Measuring the complete set of metabolites in an individual (i.e. the metabolome) provides a functional readout for cellular pathways. Further, changes in the metabolome can be correlated with disease status, prognosis and progression. Using a metabolomics platform and machine learning algorithms, biomarker signatures can be identified to predict response to therapy. Metabolite analysis of pretreatment plasma has shown **promise for predicting response** in a small retrospective study of the CDK4/6 inhibitors palbociclib and ribociclib in hormone receptor-positive metastatic breast cancer (Zhang B et al, ASCO 2019 Abstr 3043). Due to the prominent role of HER2 signaling in metabolism, we hypothesized that a metabolite signature exists to correlate with trastuzumab response.

METHODS:

Pretreatment serum from 36 HER2+ trastuzumab-naive metastatic breast cancer patients who were treated with trastuzumab were included in this exploratory analysis. Metabolites were extracted from previously frozen serum (1 mL) using ice-cold methanol. The resulting metabolites were isolated and quantified using an unbiased, non-destructive, nuclear magnetic resonance (NMR)-based profiling platform (Olaris, Inc., Cambridge, MA). The serum was analyzed via 1D ^{1}H NMR and 2D ^{13}C-^{1}H heteronuclear single quantum coherence spectroscopy (HSQC) using customized non-uniform sampling (NUS) techniques and processed with proprietary Olaris software. Supervised and unsupervised machine learning algorithms were used to identify patients with shorter and longer PFS to trastuzumab-based therapy.

The Olaris Biomarker of Response (BoR) Platform



NMR | Machine Learning | Link to Biology

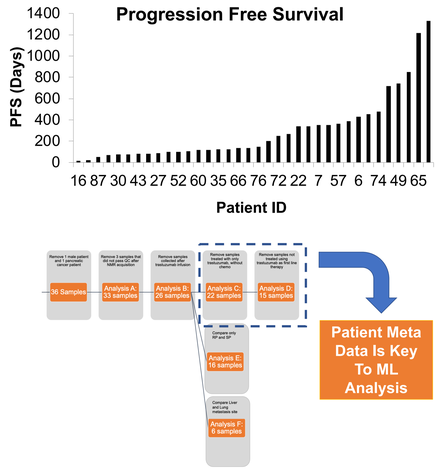
Using our patent-pending NMR-based metabolomics platform we detect and quantify **metabolites** from patient biofluids (blood or urine). Using statistics and **machine learning** we identify **"biomarkers of response" (BoR)** that are able to differentiate NR vs R. Further, we link signatures back to **biology** to uncover new insights into disease mechanisms.

RESULTS:

The patient meta data for the 36 HER2+ mBC patients treated with trastuzumab is provided in the Table below. PFS was calculated as the time from the start of trastuzumab treatment until the time of cancer progression or death. **Response (R) or Non-response (NR) to trastuzumab was determined as a PFS above (R) or below (NR) the median PFS of 141 days.** Line describes whether patients received trastuzumab as 1st, 2nd, 3rd or 5th line therapy. "Treatment" details the treatment regime for each patient where 1=single agent trastuzumab, 2=+vinorelbine, 3=+vinorelbine+cisplastin, 4=+epirubicin+doxetaxel, 5=+docetaxel, 6=+paciltaxel, 7=+taxol+gemzar, 8=+caelyx, 9=+Xeloda, 11=+taxotere+gemzar, 12=+FEC, 13=+FLEP, 14=+CIS/GEMZar, 15=+Xeloda+vinorelbine, 16=+gemzar. "Collection" describes whether serum was taken before or after trastuzumab infusion.

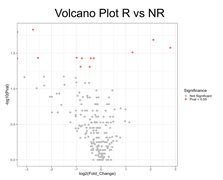
Patient Meta Data

Patient ID	PFS (days)	R or NR	Line	Treatment	Collection
16	14	NR	3	2	Before
13	22	NR	2	1	After
67	50	NR	1	1	Before
21	71	NR	2	6	Before
30	77	NR	1	2	Before
25	78	NR	1	6	Before
43	79	NR	1	7	After
41	83	NR	2	2	Before
27	89	NR	1	2	Before
3	98	NR	1	2	Before
52	98	NR	1	2	Before
64	103	NR	1	2	Before
60	117	NR	1	3	Before
45	118	NR	1	2	Before
35	124	NR	1	1	After
48	126	NR	1	1	Before
66	134	NR	2	5	Before
46	137	NR	2	2	Before
9	146	R	3	2	After
88	202	R	1	4	After
72	249	R	2	2	Before
53	270	R	1	2	Before
22	338	R	1	5	Before
12	342	R	1	1	Before
7	352	R	5	6	Before
1	353	R	1	2	Before
57	364	R	1	5	Before
71	389	R	2	1	Before
6	431	R	1	2	Before
33	455	R	1	5	Before
74	477	R	2	9	After
73	717	R	1	2	After
49	739	R	1	2	Before
51	851	R	1	2	Before
65	1217	R	1	1	Before
11	1331	R	1	2	After



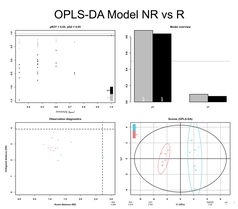
We performed a total of **6 analyses** for this study. In ***Analysis A*** we compared all NR (N=17) vs R (N=16) in the 33 patient samples passing QC. There was minimal discrimination between R vs. NR. Previous reports have suggested that blood collection methods strongly influence metabolite levels. For this reason, we removed all samples that were collected "After" (N=7) trastuzumab infusion. For ***Analysis B*** we compared NR (N=14) vs R (N=12) for the remaining 26 patients. The ability to differentiate R vs NR was still limited. We re-examined the patient meta data and observed significant heterogeneity in the treatment regime and treatment line. For example, a few patients received trastuzumab as a mono-therapy, while the remaining received trastuzumab with chemotherapy. Further, several patients received trastuzumab as part of 1st line therapy, while others received it as 2nd, 3rd or 5th line. For ***Analysis C***, we compared NR (N=12) vs R (N=10) for the 22 patients who received trastuzumab as part of combination therapy. We then focused on a subset of these patients in ***Analysis D*** (N=15), who received trastuzumab + chemotherapy in 1st line treatment. The results of Analysis C and D provided promising results. We also performed 2 independent analysis comparing 1) the patients with the extreme outcomes, Rapid Progressors (N=14) vs Slow Progressors (N=2) in ***Analysis E*** and 2) comparing patients with Lung (N=4) vs Liver (N=2) metastasis in ***Analysis F.***

Differential Metabolite Resonance Grids for NR vs R for Trastuzumab + Chemotherapy 1st Line



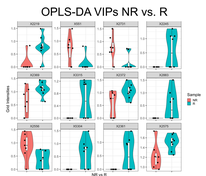
p-value <0.05	NR vs R
Significant changes	18
Increased	3
Decreased	15
*Expected by chance	12

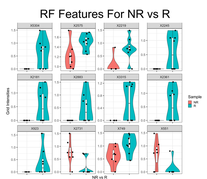
Predictive Models for NR vs R for Trastuzumab + Chemotherapy 1st Line



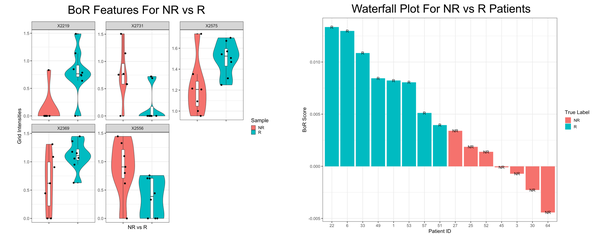
RF Confusion Matrix NR vs R

		Truth	
		NR	R
Prediction	NR	7	0
	R	0	8





Olaris BoR Differentiates NR vs R for Trastuzumab + Chemotherapy 1st Line

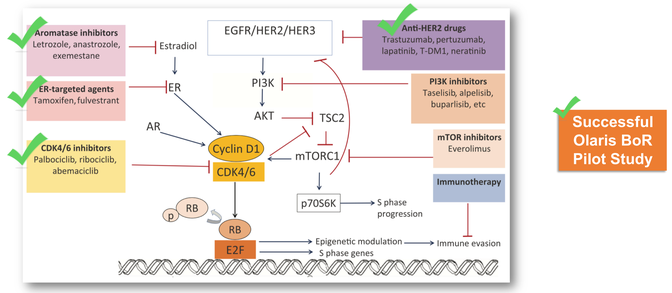


CONCLUSIONS & NEXT STEPS:

Using proprietary machine learning we were able to construct a model based on 5 metabolite resonances that could differentiate NR vs R in mBC patients receiving trastuzumab + chemotherapy as first-line treatment. Further efforts are underway to confirm the identity of these metabolites. **Expanded metabolome analysis is warranted in larger cohorts and clinical trials to confirm that this serum biomarker signature predicts PFS to trastuzumab therapy, particularly in the first-line setting.** Further, by identifying the metabolites and metabolic pathways that differ between early and late progressors, it may be possible to identify novel targets and/or suggest combination treatments in the HER2+ metastatic breast cancer setting. In fact when we compared the patients with the extreme outcomes, Rapid Progressors "RP" (N=14) were patients with a PFS less than 150 days and Slow Progressors "SP" (N=2) were those patients with PFS greater than 700 days, we identified 104 metabolite resonances grids that differed between RP vs SP with a p-value < 0.05. 92 of these grids passed the false discovery rate (FDR) and only 18 grids were expected by chance with this cut-off. This could suggest there is a significant metabolic difference between patients who have the best and worst response to trastuzumab. Additional samples will be required to verify these results.

Olaris' BoR Can Transform Breast Cancer Treatment

Current Breast Cancer Drug Treatment Options



Olaris is founded on the belief that **collaborative** research will lead to breakthrough science with the potential to transform human health. We believe in a future where every drug includes a BoR to empower optimal treatment decisions. Let's collaborate to make this a reality!

For more information please contact: EODAY@OLARISBOR.COM
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