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CLINICAL TRIAL

A pilot study utilizing multi-omic molecular profiling to find potential targets and select individualized treatments for patients with previously treated metastatic breast cancer

Gayle S. Jameson · Emanuel F. Petricoin · Jasgit Sachdev · Lance A. Liotta · David M. Loesch · Stephen P. Anthony · Manpreet K. Chadha · Julia D. Wulfkuhle · Rosa I. Gallagher · Kimberley A. Reeder · Mariaelena Pierobon · Monica R. Fulk · Nina A. Cantafio · Bryant Dunetz · William D. Mikrut · Daniel D. Von Hoff · Nicholas J. Robert

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Abstract The primary objective was to determine if multi-omic molecular profiling (MMP) informed selection of approved cancer treatments could change the clinical course of disease for patients with previously treated metastatic breast cancer (MBC) (i.e., produce a growth modulation index (GMI) $\geq$1.3). GMI was calculated as the ratio of progression free survival on MMP-selected therapy/time to progression on last prior treatment. To meet the primary objective at least 35 % of the subjects should demonstrate a GMI $\geq$1.3. Secondary endpoints included determining the response rate (according to RECIST 1.1), the percent of patients with non-progression at 4 months, and overall survival in patients whose therapy is selected by molecular profiling and proteomic analysis. Eligible patients had MBC, with $\geq$3 prior lines of therapy. A multi-omic based approach was performed incorporating multiplexed immunohistochemistry, c-DNA microarray, and phosphoprotein pathway activation mapping by reverse phase protein array. MMP was performed on fresh core biopsies; results were generated and sent to a Treatment Selection Committee (TSC) for review and treatment selection. Three sites enrolled 28 patients, of which 25 were evaluable. The median range of prior treatment was 7 (range 3–12). The MMP analysis and treatment recommendation were delivered within a median of 15.5 days from biopsy (range 12–23). The TSC selected MMP-rationalized treatment in 100 % (25/25) of cases. None of the MMP-based therapies were the same as what the clinician would have selected if the MMP had not been performed. GMI $\geq$1.3 was reported in 11/25 (44 %) patients. Partial responses were noted in 5/25 (20 %), stable disease

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G. S. Jameson (✉) · J. Sachdev · M. K. Chadha · M. R. Fulk · D. D. Von Hoff
Virginia G Piper Cancer Center - Clinical Trials, Scottsdale Healthcare, Scottsdale, AZ, USA
e-mail: GJameson@SHC.org

E. F. Petricoin · L. A. Liotta · J. D. Wulfkuhle · R. I. Gallagher · K. A. Reeder · M. Pierobon
Center for Applied Proteomics and Molecular Medicine (CAPMM), George Mason University, Manassas, VA, USA

D. M. Loesch
Caris Life Sciences, Phoenix, AZ, USA

S. P. Anthony
Evergreen Hematology & Oncology, Spokane, WA, USA

N. A. Cantafio
Translational Drug Development LLC, (TD2), Scottsdale, AZ, USA

B. Dunetz
Side-Out Foundation, Fairfax, VA, USA

W. D. Mikrut
Vantage Data Designs, Inc., San Diego, CA, USA

D. D. Von Hoff
Translational Genomics Research Institute (TGen), Phoenix, AZ, USA

N. J. Robert
Virginia Cancer Specialists, Fairfax, VA, USA

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in 8/25 (32 %) and 9/25 (36 %) had no progression at 4 months. This pilot study demonstrates the feasibility of finding possible treatments for patients with previously treated MBC using a multiplexed MMP-rationalized treatment recommendation. This MMP approach merits further investigation.

Keywords Individualized treatment · Metastatic breast cancer · Translational research · Translational oncology · Molecular profiling · Multi-omic profiling · Reverse phase protein array (RPPA)

Introduction

Breast cancer is a complex family of diseases with substantial molecular heterogeneity and varied natural history. Although progress continues to be made in the treatment of breast cancer, the majority of patients with MBC will die of their disease. Advances in targeted therapies have improved overall survival in subsets of patients (e.g., trastuzumab in HER2+ breast cancers). However, patients' tumors eventually progress and generally will require a series of consecutive treatments. With each subsequent standard treatment, a decrease in progression free survival (PFS) has been observed in advanced cancer patients [1–4].

There is no gold standard for the treatment of MBC, and currently over 30 anticancer agents are approved by the FDA for the treatment of breast cancer [5]. Although considerations like the toxicity profile of one regimen versus another, age and performance status of the patient, tumor burden, etc. play a role, the selection of treatment regimens in first line and beyond is largely empiric. Identification of specific molecular drivers of cancer, such as ER, HER2, EGFR, ALK, BRAF, KRAS, etc. in different tumor types have previously been associated with specific patient populations that are more likely to respond to target specific therapies. While testing for expression/alteration of these genes and proteins have been largely constrained to organ-specific analysis (e.g., ER/HER2 for breast cancer, BRAF for melanoma, etc.), there have been efforts to show that patients who are treated based on a more global MMP-rationalized approach, regardless of the primary site of origin of the tumor, receive clinical benefit [6, 7]. Previously, these studies have relied on single gene alterations like mutations, amplifications etc., or protein expression or lack thereof, in tumor samples for therapeutic correlation. However, there is a growing body of evidence that reveals that post-translational modifications such as protein phosphorylation can provide predictive information about treatment response, which is not revealed by genomic analysis [8–12]. Indeed, the mechanism of action of many of the FDA approved molecular targeted pathway-based therapies such as those that target EGFR, HER2, cKIT, SRC, mTOR, PDGFR, etc. is directed at the modulation of protein enzymatic activity, namely protein kinase activity, which is controlled mainly via protein phosphorylation driven signaling. Moreover, there are data emerging that protein expression and activation states are often different in the metastatic lesion compared to the primary tumor [13–18]. This provides a rationale for re-biopsy of the metastatic sites of disease to tailor treatment to the recurrent as opposed to the primary tumor.

This pilot study evaluates the feasibility and potential of prospectively combining a variety of technologies for tumor molecular profiling of metastatic disease by incorporating a "multi-omic" approach wherein protein expression, protein activation/phosphorylation, and transcriptomic analysis are used for selecting approved cancer treatments in patients with heavily pretreated and progressing MBC.

Methods

Study objectives

The primary objective was to determine the percentage of patients with refractory breast cancer where MMP-informed selection of approved cancer therapies could change the clinical course of their disease to produce a Growth Modulation Index (GMI) $\geq$1.3. The GMI was calculated as the progression free survival (PFS) on MMP-selected therapy/time to progression (TTP) on last prior treatment. A GMI of 1.3 was selected because 30 % or greater improvement in PFS with MMP-selected treatment compared to previous TTP would be considered clinically meaningful. To meet the primary objective, the GMI needed to be $\geq$1.3 in a least 35 % of study participants. Secondary objectives were to determine: frequency with which MMP yields a target against which there is a commercially available, approved agent or therapeutic regimen, percentage of time in which the treatment selected by MMP was different than that which would have been selected by the patient's physician, overall best response rate (according to RECIST 1.1) and percentage of patients with non-progression at 4 months, and the overall survival (OS).

Study design

This open-label, multicenter, pilot study accrued patients between March 2010 and June 2012. The study was conducted across three sites in the United States in accordance with International Conference on Harmonization Tripartite Good Clinical Practice Guidelines and was approved by independent Institutional Review Boards (IRBs). Patients

provided written informed consent prior to any study-related procedures being performed.

Eligibility criteria

Patients ≥18 years of age with a diagnosis of MBC who had refractory disease, were evaluated for the study. Refractory disease was defined by the following criteria: Progression of disease (PD) on ≥3 prior chemotherapeutic or biological regimens for advanced disease; PD on the last treatment. The last treatment duration must have been ≥4 weeks but ≤6 months; measurable disease by RECIST 1.1 with clear documentation of progression; and start and stop dates on the last prior treatment. Other requirements included: ECOG performance status 0–1; adequate organ and bone marrow function; off treatment for ≥3 weeks or 5× half life of drugs and have recovered from the side effects (≤grade 1) of prior regimen; be a good medical candidate for and willing to undergo a biopsy or surgical procedure to obtain tissue. As part of eligibility the treating investigator had to designate the treatment he/she would prescribe if no MMP results were available for the patient.

Study treatment

Consented patients were screened with eligibility verified by the Principal Investigator (GSJ). A disease assessment was to be performed during a study specified GMI window that was calculated and provided to the treating Investigator at the time of enrollment. The GMI window was calculated as the "window" between TTP and 1.25× TTP on last prior treatment and was required by the study in order to rule out the possibility that a GMI ≥1.3 was obtained simply because of the timing of the assessment.

Once enrolled in the study a tumor biopsy was performed. Four 18-gauge needle core biopsies were required. If adequate tumor tissue was obtained, MMP was performed. Treatment was selected on the basis of targets identified from the analysis of the patient's biopsy. A Treatment Selection Committee (TSC) comprised one oncologist/pathologist (NJR), one oncologist (DML), one oncology nurse practitioner (GSJ), one pathologist (LAL), and at minimum two bench scientists (LAL, MP, EFP), reviewed the results of the MMP and selected a specific treatment regimen following a defined algorithm as detailed in Supplemental Table I. The final recommended treatment took into account the patient's medical history including: prior treatments, co-morbidities, and drug safety profiles. When possible, a combination treatment was selected over a single agent.

In the event that no molecular target(s) was identified, the patient would be offered the treatment selected on an empirical basis by the treating physician and would be followed for survival status. Treatments were given according to the manufacturer's instructions and standard institutional practice. Only approved treatments were prescribed; although off-label use for approved oncology treatments may have been used, no investigational agents were administered.

Tumor MMP analysis

Fresh frozen (FF) and representative formalin fixed paraffin-embedded (FFPE) tissue were required. Caris Life Sciences performed the c-DNA microarray (cDNA MA) and immunohistochemistry/fluorescence in situ hybridization (IHC/FISH) analyses under CLIA (e.g., Target Now™). The Center for Applied Proteomics and Molecular Medicine (CAPMM) at George Mason University performed the Reverse Phase Protein Microarray (RPPA) analyses being developed and evaluated in accordance with College of American Pathologist (CAP) guidelines. All MMP assay results were forwarded and treatment recommendations were made by the TSC within a median of 15.5 days from the date of biopsy (range 12–23). A full description of the tumor MMP analysis can be found in the Supplemental Material II.

Assessments

Disease status was to be assessed at baseline and then every 7 ± 1 weeks and during the required GMI window (the period after treatment initiation covering the "window" between TTP and 1.25× TTP on last prior treatment), until progression or time of treatment discontinuation, according to RECIST 1.1 criteria. Adverse events (AE) were assessed at every visit and graded according to National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE) Version 3.0. For the purposes of this study, only the following adverse events were reported: grade ≥3; AE resulting in dose reduction or treatment discontinuation; AE considered medically significant by treating investigator; and any serious adverse event. Tumor markers were followed in those patients with elevated tumor markers on last prior regimen, and were assessed every 7 ± 1 week until study discontinuation or disease progression. Survival status was collected every 4 months for the first year following study discontinuation and then every 6 months until 24 months from discontinuation or study closure.

Statistical considerations and methods

The sample size was determined according to the exact single-stage design for phase II studies, using a type I error rate of 5 % (one-sided) and a power of 90 %. It is taken

Table 1 Patient demographics and clinical characteristics

Characteristic	*n*	%
Gender		
Female	28	100
Age (years)		
Median	58	
Range	33–77	
Ethnicity		
Not of Hispanic/Latin origin	26	93
Hispanic/Latin origin	2	7
Race		
Asian	1	3.5
White	26	93
Unknown	1	3.5
ECOG PS		
0	16	57
1	12	43
Metastatic at diagnosis	6	21
Site of metastases		
Abdomen/peritoneum	4	14
Bone	18	64
Colon	2	7
CNS	3	11
Liver	18	64
Lung/pleura	10	36
Lymph nodes	13	46
Ovary	2	7
Skin	11	39
Other	9	32
Number of prior treatment regimens (range 3–12)		
3	1	3.5
4	4	14
5	4	14
6	3	11
7	4	14
8	5	18
9	4	14
10	1	3.5
11	1	3.5
12	1	3.5
Original tumor characteristics		
ER		
Positive	24	86
Negative	4	14
PR		
Positive	15	54
Negative	13	46
Her2		
Positive	5	18
Negative	22	79
Unknown	1	4
BRCA-2	3	10

ECOG PS Eastern Cooperative Oncology Group performance status, *ER* estrogen receptor, *PR* progesterone receptor, *Her2* human epidermal growth factor receptor 2, *BRCA-2* breast cancer type 2 susceptibility protein

that $\leq$10 % of patients with GMI $\geq$1.3 is an uninteresting outcome and that $\geq$35 % is an outcome that is considered promising. According to this design, 25 evaluable patients were needed for the study. If after 25 evaluable patients, $\geq$6 responders have a GMI $\geq$1.3, the study treatment allocation method would merit further investigation in the previously treated MBC population.

Results for the primary objective—evaluation of GMI—were presented with descriptive statistics as the ratio of PFS on current therapy over the PFS on latest therapy. The percentage of patients with GMI $\geq$1.3 was displayed along with its corresponding 95 % exact confidence interval.

All other statistical analyses for response and stable disease were mainly descriptive. Continuous variables were summarized using number of events (*N*), mean, standard deviation, median, minimum and maximum; categorical variables were presented using frequencies and percentages; and time-to-event variables were described by *N*, median, range, number censored, and Kaplan–Meier plots.

Results

Patient demographics and disposition

A total of 28 female patients were enrolled and underwent a tumor biopsy for the purposes of this study. 25/28 patients were treated on study with the MMP-selected treatment and were evaluable for the primary end point of GMI. Of the three patients that did not receive study-selected treatment, two patients had rapid disease progression following the biopsy and chose palliative care, and one patient elected to receive treatment off study. The patient demographics and characteristics are detailed in Table 1. This was a heavily pretreated population with 20 % of patients having metastatic disease at initial diagnosis and a median number of seven prior treatments (range 4–12). As of March 2014 the study was closed at which time two patients continued on study-selected treatment, 22 had discontinued study-selected treatment

Table 2 Tumor characteristics, GMI, method of analysis, targets identified, and selected treatment

Subject ID	Baseline HR/ HER2 Status	Number of prior treatments	Study Biopsy Site	Actual Patient GMI	TTP on study treatment (days)	TTP on last line of therapy (days)	Method of analysis	Targets used to select treatment and method used	Prior drug regimen	Selected treatment based on Pt's tumor MMP	Change in HR/HER2 from original DX
100	HR+ HER2+	6	Liver	0.459	56	122	I, R	$HER2^{I}$, ER^{I}, TS^{I},	Ixabepilone + lapatinib	Lapatinib + capecitabine	No change
101	*HR+ HER2−*	*9*	*Scalp*	***1.977***	*87*	*44*	*I, M, R*	*$TOPO1^{I}$, $TOP1^{M}$*	*Vinorelbine*	*Irinotecan*	*No change*
102	HR− HER2−	8	Chest Wall	0.465	20	43	I, F, M, R	$HER2^{I}$, $ERBB2^{M}$, $PTEN^{I,M,R}$	Sunitinib + CVX-060	Lapatinib + traztuzumab	HER2+
103	HR− HER2−	9	Pelvis	0.571	40	70	I, F, M, R	ER^{I}, PR^{I}, AR^{I}, $PTEN^{I,R}$, $EGFR^{F,R}$	Vinorelbine	Erlotinib + letrozole	ER+, PR+
104	HR+ HER2−	8	Liver	0.236	38	161	I, R	TS^{I}	Doxorubicin + cyclophosphamide	Capecitabine	No change
105	HR+ HER2−	4	Chest Wall	0.319	15	47	I, F, M, R	$TOPO1^{I}$, $TOP1^{M}$	Bevacizumab + gemcitabine	Irinotecan	No change
106	HR+ HER2−	3	Liver	NA[a]	NA[a]	100	I, F	$SPARC^{I}$	EZN-2208 (PEG-SN38)	Nab-paclitaxel	HER2 -
107	*HR+ HER2−*	*9*	*Liver*	***1.303***	*86*	*66*	*I, F, R*	*$TOPO1^{I}$, TS^{I}*	*Gemcitabine*	*FOLFIRI*	*No change*
108	HR+ HER2−	8	Breast	1.181	196	166	I, F, M, R	$TOP2A^{I,M}$, PGP^{I}	EZN-2208	Liposomal doxorubicin	No change
109	*HR+ HER2−*	*12*	*Liver*	***7.156***	*1238+*	*173*	*I, F, M, R*	*$TOP2A^{I,M}$, $HER2^{I,F}$, $PTEN^{I,R}$, $ERBB2^{R}$*	*Vinorelbine*	*Irinotecan + trastuzumab*	*HER2+*
110	*HR+ HER2+*	*6*	*Axilla*	***6.873***	*378*	*55*	*I, F, M, R*	*$TOP2A^{I,M,F}$, PGP^{I}, $HER2^{I,F}$, $PTEN^{I}$, TS^{I}, $TYMS^{M}$, $TOPO1^{I}$, $PIK3A^{F}$*	*Vinorelbine*	*Liposomal doxorubicin → doxorubicin → FOLFIRI*[b]	*No change*
111	HR+ HER2−	8	Supraclavicular Node	0.857	36	42	I, F, M, R	$TOP2A^{I,M}$, PGP^{I}	Nab-paclitaxel	Liposomal doxorubicin	No change
112	HR+ HER2−	5	Omentum	0.046	9	196	I, M	$TOPO1^{I}$, TS^{I}	Epirubicin + cyclophosphamide	FOLFIRI	No change
113	*HR+ HER2−*	*4*	*Posterior Shoulder*	***2.260******	*113*	*50*	*I, F, M*	*ER^{I}, $ESR1^{M}$, PR^{I}, $TOPO1^{I}$*	*Gemcitabine + paclitaxel*	*Exemestane + irinotecan*	*No change*
114	HR+ HER2−	8	Liver	0.729	62	85	I, F, M, R	ER^{I}, $ESR1^{M}$, PR^{I}	Gemcitabine + capecitabine	Letrozole	No change
115	*HR+ HER2−*	*5*	*Breast*	***1.684***	*165*	*98*	*I, F, M, R*	*ER^{I}, $ESR1^{M}$, $PR^{I,M}$, TS^{I}, $TYMS^{M}$, $TOPO1^{I}$*	*Gemcitabine + capecitabine*	*Letrozole → irinotecan + fluorouracil*	*No change*
116	*HR−HER2−*	*7*	*Liver*	***2.778***	*275*	*99*	*I, F, M, R*	*$SPARC^{I}$*	*Gemcitabine*	*Nab paclitaxel*	*HR+*
117	*HR+ HER2−*	*9*	*Liver*	***3.408***	*351*	*103*	*I, F, M, R*	*$TOPO1^{I}$, $TOP1^{M}$*	*Capecitabine*	*Irinotecan*	*No change*
118	*HR+ HER2−*	*6*	*Liver*	***2.527***	*235*	*93*	*I, F, R*	*$TOPO1^{I}$, TS^{I}*	*Paclitaxel*	*FOLFIRI*	*No change*
119	*HR+ HER2+*	*5*	*Liver*	***1.977***	*85*	*43*	*I, F, M, R*	*$RRM1^{I,M}$, $RRM2B^{M}$, $JRT2^{I,F}$, $PTEN^{I}$, $PIK3CA^{I}$*	*Lapatinib + capecitabine*	*Gemcitabine + trastuzumab*	*No change*
120	HR+ HER2−	11	Cervical Lymph Node	0.170	29	171	I, F, M, R	AR^{I}	Exemestane	Flutamide	No change
121	HR+ HER2 unk	7	Axillary Lymph Node	0.235	20	85	I, F, M, R	EGFR/AKT/ERK pathway $activated^{R}$ $PTEN^{IR}$, $EGFR^{I}$, $PDGFRA^{M}$	Letrozole	Erlotinib	No change
122	HR− HER2−	4	Left Flank	NA[a]	NA[a]	36	I,F,M,R	$TOPO1^{I}$, TS^{I}	Iniparib	FOLFIRI	No change

Table 2 continued

Subject ID	Baseline HR/ HER2 Status	Number of prior treatments	Study Biopsy Site	Actual Patient GMI	TTP on study treatment (days)	TTP on last line of therapy (days)	Method of analysis	Targets used to select treatment and method used	Prior drug regimen	Selected treatment based on Pt's tumor MMP	Change in HR/HER2 from original DX
123	HR+ HER2−	10	Liver	NA[a]	NA[a]	157	I,F,R	TOPO1[1]	Doxorubicin + cyclophosphamide	FOLFIRI	No change
124	*HR+ HER2+*	*4*	*Skin Nodules Breast Area*	***2.783***	*167*	*60*	*I, F, M, R*	*EGFR/AKT/ERK pathway activated*[R], *PTEN*[IR], *ER*[I], *ESR1*[M]	*Gemcitabine*	*Erlotinib + letrozole*	*No change*
125	*HR+ HER2+*	*7*	*Liver*	***2.622****	*97*	*37*	*I, F, R*	*HER2*[I,F], *PTEN*[I], *TELE3*[I], *SPARC*[I]	*Doxorubicin*	*Lapatinib + paclitaxel*	*No change*
126	HR+ HER2−	5	Liver	0.448	56	125	I, F, R	TOPO1[1]	Eribulin + ramucirumab	Irinotecan	No change
128	*HR+ HER2−*	*7*	*Breast*	***9.734***	*623+*	*64*	*I, F, R*	*TOPO1*[I], *TS*[I]	*Eribulin*	*XELIRI*	*No change*

MMP multi-omic molecular profiling, *I* immunohistochemistry, *F* fluorescence in situ hybridization, *M* microarray, *R* RPPA, *FOLFIRI* irinotecan + 5FU + folinic acid, *XELIRI* capecitabine + irinotecan; *unk* unknown

Bold Italics represents positive outcome with GMI ≥1.3

By IHC analysis, ALL patients' tumor samples demonstrated low or absent TS, normal PTEN, increased TOPO1 and MRP1

[a] NA—Not applicable as patient did not start study treatment

[b] Due to intolerance of liposomal doxorubicin, then reaching lifetime max of anthracycline, regimen changed to FOLFIRI based on MP results

[c] Due to intolerance of letrozole after 17 days, treatment changed to FOLFIRI

* Patient did not have scan completed within GMI window and therefore is not considered a GMI responder

due to disease progression and one patient was non-compliant and discontinued study treatment on her own.

Efficacy

A total of 28 patient biopsies were submitted and MMP yielded a target in all specimens that met the minimum tissue requirements for analysis. Table 2 details MMP of each tumor specimen. Nine patient specimens were inadequate for c-DNA MA (seven due to insufficient RNA yield, two due to FF specimens not being submitted). Mid-study, the c-DNA MA was optimized to be performed on FFPE tissue. Additionally, three biopsies were inadequate for RPPA analysis due to lack of tumor epithelium in the biopsy sample. Table 3 summarizes the RPPA results.

The MMP-selected treatment was different in 100 % of patients (25/25) when compared to empiric choice made by treating investigator at time of enrollment (refer to Supplemental Material Table V). Two patients had a change in treatment during the course of the study, with subsequent treatment also based on MMP results as determined by the TSC. In patient 110 this was due to intolerance of liposomal doxorubicin, so treatment was changed to doxorubicin. Once the lifetime maximum dose of anthracycline was reached, the regimen was then changed to FOLFIRI (of note, this patient did exceed the GMI while on initial anthracycline therapy). In patient 115 due to unacceptable grade 2 fatigue and myalgias on letrozole (17 days), the treatment was changed to FOLFIRI.

Of the 25 patients treated with the MMP-selected treatment, 11 of 25 were evaluated during the protocol required GMI window and demonstrated a GMI ≥1.3. Two additional patients had a GMI ≥1.3 but did not have the scans completed during the specific GMI window, and therefore could not be included as GMI responders per protocol (refer to Table 2; Fig. 1).

22/25 patients were evaluable for response by RECIST 1.1 criteria, 21 by radiological exams and 1 by physical exam. Three patients had clinical progression prior to re-staging of disease. Maximum percent change of summed diameters of target lesions is summarized in Fig. 2. Best overall responses included: five partial responses (PR), eight stable disease (SD), and nine progressive disease (PD). 9/25 (36 %) of patients had non-progression at four months. Follow-up data were collected up until March 2014, at that time five patients were alive. Median survival from start of study treatment is 7.8 months (range 0.56–40.67) refer to Supplemental Fig. II. The median survival duration of 11 patients with GMI ≥1.3 was 10.32 months (95 % CI 7.46–NA), compared with 3.88 months (95 % CI 1.97–8.35) for 12 patients with GMI <1.3 ($p = 0.06$) refer to Supplemental Fig. III.

Table 3 RPPA results

Drug target(s)	EGFR Y1173	Erb2 Y1248	VEGFR Y996	PDGFR Y751, cKit Y719, cAbl T735	mTOR S2481
Downstream	ERK	ERK	ERK	ERK	P70SKT389
Substrate from drug target	T202/Y204, AKT S473	T202/Y204, AKT S473	T202/Y204, AKT S473	T202/Y204, AKT S473	
Number of pathway activated positive patients	13/25 (52.0 %)	3/25 (12.0 %)	0	3/25 (12.0 %)	0

RPPA was not performed on 3 of 28 patients (106, 112, and 113) due to inadequate material

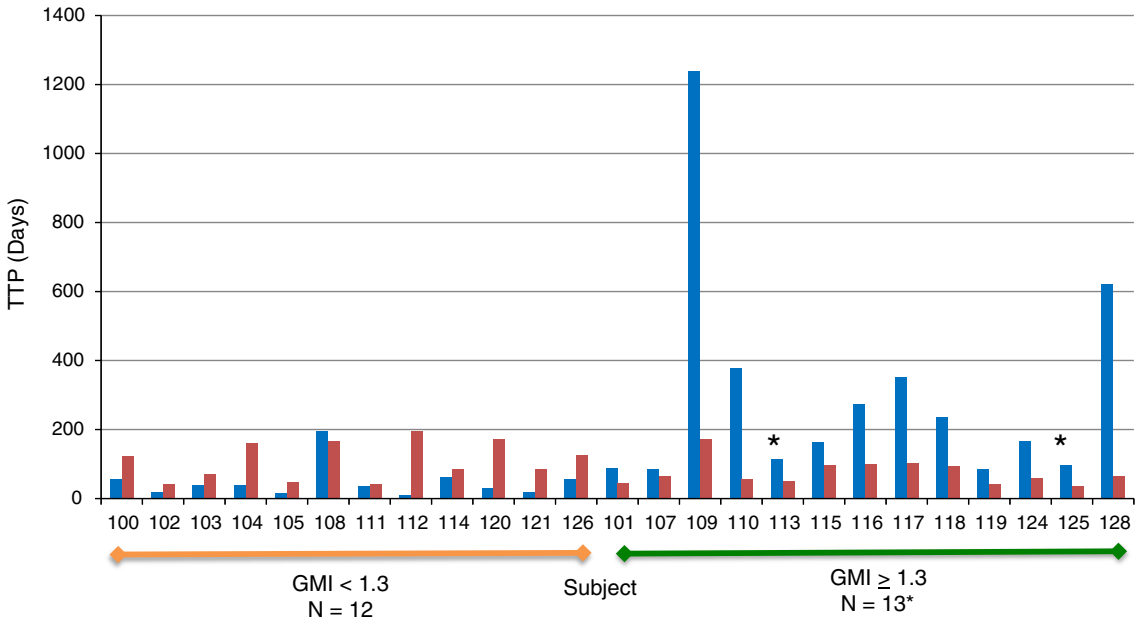

Fig. 1 Comparison of TTP on MMP-selected treatment versus prior treatment for all enrolled and treated patients ($N = 25$) *Patients 113 and 125 although they had a GMI $\geq$1.3, they did not have scan completed within GMI window, and therefore are not considered GMI responders. Therefore, GMI responders $N = 11/25$ (44 %)

Safety

All treatment-related AEs reported for this study were consistent with known AEs of these approved drugs. A total of thirteen treatment-related AEs were reported, ten of which resulted in an interruption in treatment or dose reduction and included the following: hand-foot syndrome ($n = 3$), vomiting ($n = 2$), anemia ($n = 1$), hyponatremia ($n = 1$), bone marrow toxicity ($n = 1$), *Staphylococcus aureus* infection ($n = 1$), ventricular systolic dysfunction ($n = 1$), nausea ($n = 1$), fatigue ($n = 1$), and diarrhea ($n = 1$). There were no treatment-related deaths observed on study. Two serious adverse events related to the tumor biopsy were reported in two patients that underwent a biopsy of a metastatic liver lesion. One patient experienced a hematoma requiring hospitalization, which resolved without further complications. The second patient experienced grade 3 right upper quadrant pain post biopsy and was admitted for pain control. CT scan showed no evidence of hematoma and the pain resolved.

Discussion

Our prospective multi-center study concluded the following: It is feasible to coordinate the MMP of small amount of biopsy tissue across multiple laboratories in different parts of the United States; MMP results can be generated within a 2–3 week time period; the treatment selected based on MMP in this study was different than what the physician would have chosen empirically in all (25/25) patients; and using this MMP-rationalized treatment recommendation resulted in a GMI $\geq$1.3 in 11 of 25 (44 %) heavily pretreated MBC patients.

There are some important limitations of this study that we consider here. Treatments included only approved,

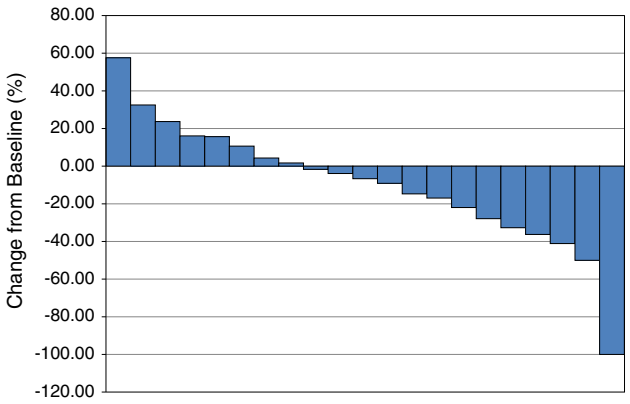

Fig. 2 Maximum percent change of summed diameters of target lesions with respect to baseline diameters by RECIST 1.1 criteria 22/25 patients were evaluable by RECIST 1.1 criteria, 21 by radiological exams and 1 on physical exam. Three patients had clinical progression prior to restaging of disease

commercially available chemotherapeutic and/or pathway-based molecularly targeted agents; investigational agents were prohibited. On several occasions RPPA results suggested the potential benefit of a PI3 kinase or ERK inhibitor, which at the time of this study were only available in clinical trials. Additionally, the recommendation of a drug for off-label treatment such as erlotinib was challenging to obtain in a timely and affordable fashion. Another limitation of the study was related to the timing of required disease assessments. In order for patients to be considered a GMI responder, the protocol required that the timing of the scans be done within the calculated GMI window. This was built into this study in order to reduce ascertainment bias. However, because the cost of scans was not covered by the study and patients were permitted to be treated by their local oncologist, scans were not always completed during this GMI window. In fact there were two such cases in which the patient met the GMI endpoint of $\geq$1.3 but did not have a scan done during the GMI window and the next subsequent scan demonstrated disease progression. Among the 23 patients who did undergo scans within the protocol-specified GMI window, the proportion of patients achieving a GMI $\geq$1.3 was 48 % (11/23).

Important molecular observations of this study, consistent with other studies include: a change in HER2 status ($n = 2$) and hormone receptor (HR) status ($n = 2$), which was important in selecting treatment and demonstrates the value of biopsy at the time of progressive disease. By IHC analysis all samples demonstrated low or absent thymidylate synthase (TS), normal phosphatase and tensin homolog (PTEN), increased topoisomerase 1 (TOPO1) and multidrug resistant associated protein-1 (MRP1). 14 of 25 patients were treated with irinotecan and/or fluorouracil based regimens. Of note, TOPO1 inhibitors including irinotecan and etirinotecan (under study) have demonstrated antitumor activity in MBC clinical trials, although none have been approved to date as standard treatment for MBC [19, 20]. There are no prospective published data to support the use of TOPO1 as a predictive biomarker for the efficacy of irinotecan in MBC. In light of this, our trial results do provide a valuable hypothesis, at least within the context of MBC, of TOPO1 being able to predict for irinotecan efficacy and the need for further investigation and validation of this marker. The randomized phase 3 BEACON trial of etirinotecan versus physicians' choice chemotherapy in MBC is evaluating TOPO 1 among other biomarkers in circulating tumor cells from patients on the study and will hopefully provide additional validation of this target for treatment with topoisomerase inhibitors [21].

When available, RPPA results were considered in all cases and led the treatment selection in 3 patients. For analytes measured by both IHC and RPPA (PTEN and HER2) both were 100 % concordant for HER2 and PTEN and phospho HER2–HER2 concordance was observed in all cases. The c-DNA MA data played a lesser role in treatment recommendations and in many cases supported the IHC data (refer to Table 2), both the c-DNA MA and RPPA data were used to de-prioritize potential therapies that otherwise could have been selected in the absence of MMP.

As multi-omic technology improves and more targets are discovered, presumably more treatment options will be available, increasing the likelihood of improving outcomes in MBC. If this approach were coordinated with drug development so that investigational agents are also available for treatment, this possibly would increase the number of options for the patient. There is precedent in a study sponsored by Stand Up 2 Cancer that will be conducted in BRAF wild type melanoma patients, in which patients will have access to investigational drugs identified as potential therapeutic targets based on profiling of patient biopsy tissue [22].

In summary, the major strength of our study is the successful prospective application of a first-of-its-kind highly multiplexed MMP-rationalized treatment approach with real-time availability of results (within 15.5 days of the fresh biopsy) in the treatment of MBC. This pilot study met its primary clinical endpoints as 44 % of all patients achieved GMI $\geq$1.3. Although not a significant difference, a positive trend emerged between GMI response and survival ($p = 0.06$) that is worth further exploration in future studies.

These data support the results of a precedent study, wherein 44 % of the subset of MBC patients ($n = 18$), had a PFS ratio of $\geq$1.3 on an MMP-suggested regimen than on the regimen on which the patient had just experienced disease progression [2]. While this current study was not

designed as a definitive trial for any specific therapy for MBC and did not have sufficient power for validation of the predictive effect of any specific biomarker-drug pair, certain biomarkers like TS and TOPO1 were predominant in treatment selection (Table 2). Further, across the population of patients and in the face of the heterogeneous molecular landscape observed, our multi-omic approach resulted in molecularly rationalized treatment options that were used in 100 % of the patients. Our approach to these biomarker guided treatment recommendations is considered relevant within the scope of this study and will need to be evaluated in future adequately powered biomarker driven studies prior to concluding their predictive utility for specific treatments.

MMP-rationalized treatment has the potential to improve patient outcomes, while avoiding unnecessary toxicity from treatments not supported by the patient's tumor biology. A randomized prospective trial comparing MMP-based treatment with physician's choice is needed to demonstrate if it is a more effective approach.

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Conflict of interests Gayle Jameson reports grants from Side-Out Foundation and serves in a Consultant/Advisory role with Celgene. Dr. Petricoin reports grants from Side-Out Foundation, during the conduct of the study; University, personal fees from Perthera, Inc., outside the submitted work; In addition, Dr. Petricoin has a patent Methods for the Isolation and Analysis of Cellular Protein Content 6,969,614 with royalties paid, and a patent mTOR pathway Theranostic US8628931 B2 pending and Stock ownership: Theranostics Health, Inc. and Pethera, Inc. Dr. Sachdev reports grants from Celgene, grants from Pfizer, drug support from Genentech and advisory board payment from Celgene outside the submitted work. Dr. Liotta reports personal fees from Theranostics Health, Inc., outside the submitted work; and stock ownership: Theranostics Health. NIH royalty fees received for Analysis of Cellular Protein Content6,969,614, and Laser Capture Microdissection 5,843,644; 5,843,657;6,010,888; 6,204,0030; 6,251,516. Dr. Loesch reports personal fees from Caris Life Sciences, during the conduct of the study. Dr. Anthony reports personal fees from Caris Life Sciences, during the conduct of the study. Dr. Chadha declares that she has no conflict of interest. Dr. Wulfkuhle reports Stock Ownership: Theranostics Health. Rosa Gallagher reports grants from Side Out Foundation, during the conduct of the study. Kimberley Reeder reports grants from Side-Out Foundation, during the conduct of the study. Dr. Pierobon reports grants from Side-Out Foundation, during the conduct of the study; Stock Ownership: Theranostics Health, serves in a Consultant/Advisory role with Perthera. Monica Fulk declares that she has no conflict of interest. Nina Cantafio reports personal fees from Translational Drug Development LLC (TD2), during the conduct of the study; and previous contract work with Caris Life Sciences. Brian Dunetz reports Employment Position- Chief Operating Officer Side Out Foundation, uncompensated. Dr. Mikrut reports personal fees from Translational Drug Development LLC (TD2), during the conduct of the study. Dr. Von Hoff reports personal fees from Caris Life Sciences, as Director of Clinical Research during the conduct of the study and Stock Ownership: Caris Life Sciences. Dr. Robert reports grants from Side-Out Foundation, during the conduct of the study.

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