

ABSTRACT



Drug Response Indicators Test (DRIT) for personalized anticancer chemotherapy for late stage breast cancer (BC) patients. PO Ts'o¹, SA Lesko¹, ZP Lum¹, S Deamond¹, E Shan¹, D Zhou¹, J Daniel¹, D Van Echo¹, K Tkaczuk², NS Tait², F Feldman², M Tan², W Rodgers². ¹CCC Diagnostics, LLC, Baltimore, MD, ²University of Maryland Greenebaum Cancer Center, Baltimore, MD.

DRIT is a diagnostic assay for personalized chemotherapy being developed by CCC Diagnostics, LLC (www.cccdiagnostics.com). This assay is designed to predict an individual patient's tumor response to FDA approved drugs. The technology is currently being evaluated in retrospective correlation studies for clinical validation. DRIT is performed on slides of fixed and paraffin embedded tumor tissue. The assay can quantitatively determine tumor response to a specific drug based on the expression level of Drug Response Indicators (DRI) / biomarkers. The DRI / drug relationship is initially verified in vitro with 8-10 cancer cell lines of differing drug sensitivity by correlating cytotoxicity (IC₅₀) and DRI expression (Pearson correlation coefficient 0.9, p 0.02). In patient tumors DRI quantitation is performed in 500 tumor cells based on staining with fluorescent-dye labeled monoclonal antibodies specific for drug function. Fluorescence is measured by computerized fluorescence microscopy with external calibration. Individual tumors are classified as sensitive or resistant to a drug based on the expression level of a mechanistically related DRI. In retrospective clinical correlation studies, an individual patient is classified as responder or non-responder to a particular drug regimen based on RECIST guidelines. Investigators performing the DRIT analysis were blinded as to clinical response data until correlation between the clinical outcome (responder/non-responder) versus DRI level (sensitive/resistant tumor) was evaluated for each drug treatment

ABSTRACT (cont'd)



The drugs and their mechanistically related DRI used in this study are: anthracycline/topoisomerase II; capecitabine/thymidylate synthase; docetaxel, paclitaxel, abraxane/ β -tubulin isoform III, trastuzamab/HER-2; tamoxifen, aromatase inhibitor/estrogen receptor. Among 70 stage IV BC patients, drugs for first to third line treatments were administered as monotherapy in 55 cases. Among 42 predicted sensitive tumors, 38 patients were classified as responders and 4 as nonresponders. Among 13 predicted resistant tumors, 11 patients were classified as nonresponders and two as responders. Overall accuracy (agreement between predicted sensitivity of tumor and patient clinical response) is 49/55 or 89%. Utilizing the monotherapy correlation data, the correlation of two drug combination therapy to clinical response can be analyzed by dissecting out the predicted response to each individual drug in the combination therapy, assuming no mutual interaction. This analysis included 12 additional patient cases and 5 neoadjuvant treatments. This data increased the total number of cases analyzed for correlation to 72, with the accurate prediction in 65 cases resulting in a percentage accuracy of $65/72=93\%$. Statistical establishment of a cutoff point for the expression level of each DRI as related to the resistance/sensitivity to a specific drug is in progress.

Conclusion: Early retrospective correlative studies with late stage BC patients indicate that DRIT displays approximately 90% accuracy in predicting the outcome of treatment with FDA approved drugs. Clinical trials should be expanded in order to statistically confirm this data. (Supported in part by Maryland Industrial Partnerships (MIPS).)

Personalized Anticancer Therapy

Background & Rationale



- The future of cancer care, depends on better characterization of the tumor's sensitivity/resistance to anticancer drugs in individual patients.
- Currently very few anticancer therapies are truly targeted.
- Some examples of Targeted therapies include:
 - Estrogen receptor & antiestrogen therapy
 - Her2 & Trastuzumab therapy

DRIT Methods



- Specimens are coded and submitted for analysis
- Deparaffinized slides scanned for cytokeratin-positive cells.
- Five images are acquired from different areas of each tumor.
- Antibodies directly labeled with Alexa dyes are used for staining
- Fluorescence signals analyzed by computerized fluorescence microscopy
- DRI expression in the tumor sections is measured in up to 500 tumor cells utilizing 5 digital images
- Potential sensitivity or resistance is based on level of comparative DRI expression and comparison with an in vitro guideline
- Resistance versus sensitivity is established from in vitro studies.

Drug Response Indicators Breast Cancer



- Drugs - FDA approved, NCCN recommended

DRUG	DRI
Trastuzumab	HER-2/neu
Antiestrogens	ER
Taxanes/ Vinca Alkaloids	B-TUB III
5 FU	TS
Antracyclines	TOPO IIa
Platinum Salts	ERCC-1

DRI / Drug Qualification



- All DRI are qualified by an in vitro reference system
- Statistical Correlation- in vitro IC₅₀ / DRI
- Pearson ranking correlation coefficients (>0.8 / p value <0.05).
- Positive linear correlation when DRI = target of the drug
- Negative correlation when DRI represents the interference component of the drug action.

DRUG	DRI	PEARSON CORRELATION COEFFICIENT	p value
Herceptin	HER-2/neu	-0.94	0.005
Tamoxifen	Estrogen Receptor	-0.97	0.02
Docetaxel	Beta tubulin III	0.98	0.001
Paclitaxel	Beta tubulin III	0.99	0.001
5 -Fluorouracil	Thymidylate Synthase	-0.92	0.008

Retrospective DRIT Study **Breast Cancer Patients**



Eligibility Criteria

- University of Maryland IRB approved protocol/consent
- Stage 3 and 4 Breast Cancer Patients
- Mono or doublet therapy
- First three lines of therapy
- Neoadjuvant Therapy
- Excluded all experimental drug treatments
- Excluded all triplet therapy
- Excluded adjuvant Therapy and 1 cycle treatments

Patient Characteristics

Retrospective Breast Cancer Study



<u>SEX RATIO</u> Male:Female	59/2
<u>RACE</u>	
African American	43
Caucasian	15
Asian	3
<u>AGE</u>	
Male	68 (58,78)
Female	53 (36-85)
<u>STAGE – STUDY ENTRY</u>	
Stage III	8
Stage IV	53
Recurrant NED	3

DRI Expression Level Distribution

- Distribution of expression levels in sensitive vs resistant tumors are bimodal and well separated

DRUGS	DRI	SENSITIVE*	RESISTANT*
		MEAN DRI UNITS	MEAN DRI UNITS
TAM, Femara, Faslodex, Aromasin, Arimidex	ER	< 8	> 27
Epirubicin, Doxilrubicin, Adriamycin	TOPO IIa	< 3	> 17
Abraxone, Taxotere, Novalbine, Taxol, Docetaxel	TUB III	< 35	> 55

- Operational Unit – the value for one DRIU is 2000 F/P (fluorescence units per pixel).
- Molecular Unit – the number of IgG-fluorescent conjugate molecules bound to the stained tissue that produces a fluorescence intensity of 2000.

Correlation / Prediction Accuracy Tumor Sensitivity – Breast Cancer

- # accurate predictions / # treatment interventions

THERAPY	TOTAL	LINE 1	LINE 2	LINE 3
Mono	61 / 72 (84.7%)	20 / 24 (83.3%)	23 / 29 (79.3%)	18 / 19 (94.7%)
Doublet	22 / 23 (95.6%)	17 / 18 (94.4%)	3 / 3 (100%)	2 / 2 (100%)
Combined (mono + doublet)	83 / 95 (87.4%)	37 / 42 (88.1%)	26 / 32 (81.3%)	20 / 21 (95.3%)

Correlation / Prediction Accuracy Sensitive vs Resistant Tumors

- # accurate predictions / # treatment interventions
- R = Resistant S = Sensitive

Total S	Total R	Line 1 S	Line 1 R	Line 2 S	Line 2 R	Line 3 S	Line 3 R
71 / 74	12 / 21	37 / 38	1 / 5	19 / 21	7 / 11	16 / 16	3 / 4
95 %	57 %	97 %	20 %	90 %	63 %	100 %	75 %

ER Correlation/Prediction Accuracy



DRIT vs Ventana Antibody Assay(VAA)

- DRIT offers higher % correlation accuracy than VAA
- Ventana Antibody Assay performed by U. MD.
- DRIT performed by CCC Diagnostics LLC

Procedure	Correlation Accuracy
DRIT	32/38 (84.2%)
VAA	26/38 (68 %) 3/38 uncertain

SUMMARY



- 113 treatment outcomes/samples from 61 patients
- DRIT Correlation Accuracy = 87.36 % (83/95)
- ER Correlation Accuracy = 87 % (33/38)
- Positive Prediction Accuracy = 94% (71/74)
- Negative Prediction Accuracy = 57 % (12/21)
- DRIT outperforms VAA in prediction of tumor response

Drug Response Indicator Test Future Development



DRIT as a Diagnostic Service will:

- Individualize Anticancer Therapy Selection
- Lessen exposure to ineffective anticancer therapies
- Reduce potential Side Effects to patients
- Potential for Saving Significant Treatment Costs