A Predictive Diagnostic Test for Drug Treatment Outcomes in Advanced Breast Cancer Extended to Advanced Colon Cancer for Personalized Anticancer Therapy

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PRESENTATIONS / ABSTRACTS

- K. H. Tkaczuk, N. S. Tait, O. Ioffe, M. Tan, M. Mohiuddin, S. Chumsri, D. A. VanEcho, M. J. Sutula, S. Lesko, S. Deamond, P. Ts'o; Drug Response Indicator Test (DRIT) as a predictive test for treatment outcomes in advanced breast cancer patients (ABC). Clin Oncol 27:15s, 2009 (suppl; abstr 1119) – ASCO 2009.

- N. B. Pandya, K. R. Tkaczuk, N. Tait, O. Ioffe, M. Tan, D. A. Van Echo, M. J. Sutula, S. A. Lesko, S. F. Deamond, P. O. Ts'o; A predictive test for therapeutic treatment outcomes of advanced gastrointestinal cancer patients (AGC). J Clin Oncol 27, 2009 (suppl; abstr e15079).

Personalized Anticancer Therapy

Background

- Cancer is a Highly Individualized Disease

- Breast Cancer = 184,450 new cases annually (2008)
  40,930 deaths annually (2008)

- GI Cancer = 176,420 new cases annually
  61,430 deaths annually

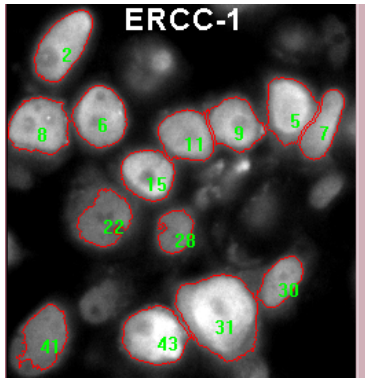
- Effective Treatment Relies on Drug Selection
  - Only 20-30% effective, significant side effects

- Some examples of Drug Response Indicator Guides include:
  - Estrogen receptor & antiestrogen therapy
  - Her2 & Trastuzumab therapy

Drug Response Indicator Test (DRIT)

- Diagnostic Service for Individual Patients
- Each DRI mechanistically related to specific drug
- Correlates DRI expression with clinical response
- Evaluates tumor response to 6 FDA approved anticancer drugs simultaneously
- Direct imaging of specified targets within a tumor tissue
- Selects effective drug treatments for Individual Patients
- Optimizes Use of Time Window for Treatment

DRIT - Methods



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6	1372	1534.849
7	951	1484.30
8	1486	1516.017
9	1185	1508.238
11	1225	1480.305
15	1171	1561.278
22	1137	1225.533
28	648	1266.161
30	823	1407.057
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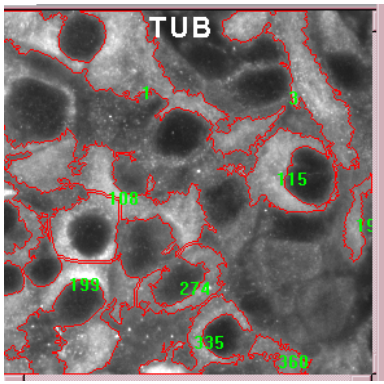

Obj.#	Area	Density Lum
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194	751	915.0293
199	7133	1071.538
274	1496	927.2640
335	1716	1033.402
360	732	860.3729

- Deparaffinized slides are subjected to antigen retrieval and stained with monoclonal antibodies.
- Antibodies directly labeled with Alexa dyes are used for staining
- Microscope calibrated to an external standard
- Stained slides are scanned to locate cytokeratin-positive cells.
- Five digital images are stored from different areas of each tumor. Also acquire images of the same fields of cells with filter cube C1 (DAPI) and C2 (cytokeratin).
- The fluorescence signals in each cropped image are analyzed by Image-Pro Plus software to select the intensities that separate the fluorescent objects (cells or cell clusters) from the background.
- IF expression in the tumor sections is measured in about 500 tumor cells utilizing 5 digital images of various areas of the tumor.
- Determinations of correlation are based on the relative level of comparative IF
- Results represented in Drug Response Indicator Units

Clinical Samples

- Clinical samples = slides cut from formaldehyde fixed, paraffin embedded breast cancer tissue
- Retrospective samples provided by:
  - University of Maryland Greenebaum Cancer Center , Baltimore, MD
  - Harborview Hospital , Baltimore , MD
- All clinical treatment outcomes determined via RECIST criteria

Drug Response Indicators

Breast Cancer

- Drugs - FDA approved, NCCN recommended

DRUG	DRI
Trastuzumab	HER-2/neu
Antiestrogens	Estrogen Receptor
Taxanes/ Vinca Alkaloids	B-TUB III
5 FU	Thymidylate Synthase
Gemcitibine	Ribonucleotide Reductase

Drug Response Indicator Units

- DRI Molecular Unit - number of IgG-fluorescent conjugate molecules bound to the stained tissue that produces a fluorescence intensity of 2000
- Operational Unit - the value for one DRIU is 2000 F/P

Antibody Conjugate	# Conjugated antibody /pixel *
Anti- Beta-Tubulin (beta chain 4) terminus III – Alexa 532	**3.3±0.09**
Anti - Estrogen Receptor mouse clone TE111.5d11 – Alexa 532	**10.2±0.25**
Anti-thymidylate Synthase clone TS 106 – Alexa 532	**8.8±0.27**
Anti – Trastuzumab – Alexa 532	**9.6±0.12**

* number of antibody conjugates in a unit area of an image that yields a fluorescence intensity of one DRIU

DRI Expression Level Distribution

Breast Cancer

- Distribution of expression levels in sensitive vs resistant

TREATMENT	DRI	SENSITIVE	RESISTANT
		MEAN DRI UNITS	MEAN DRI UNITS
Hormonal	ER	> 9.7	< 9.7
Trastuzumab	HER-2/neu	> 7.5	< 7.5
Taxanes	TUB III	< 55	> 55
Capecitabine	TS	< 25	> 25

Eligibility Criteria – Breast Cancer

- Institutional IRB approved protocol/consent
- Stage 3 and 4 Breast Cancer Patients
- Mono or doublet therapy
- First two lines of therapy
- Neoadjuvant Therapy
- Excluded all experimental drug treatments
- Excluded all triplet therapy
- Excluded adjuvant therapy and 1 cycle treatments
- Provides basis for Prospective Study now underway at The University of Maryland Cancer Center

Patient Characteristics
Breast Cancer

	UMD	Harbor	Overall
SEX RATIO Male:Female	2/50	0/14	2/64
RACE			
African American	35	2	37
Caucasian	14	12	26
Asian	3	0	3
AGE			
Male	68 (58,78)		68 (58,78)
Female	54 (36,89)	61(41,89)	56 (36,89)
STAGE – STUDY ENTRY			
Stage III	1	4	5
Stage IV	51	10	61

DRIT Prediction Accuracy

Breast Cancer

UMD / Harbor Hospital Comparison

	U MD			Harbor Hospital		
Treatment	**Sens. N=69**	**Resist**	**Predicted Response**	**Sens. N=22**	**Resist**	**Predicted Response**
Hormonal Therapy	26/29	2/2	28/31	3/3	0/0	3/3
Mono-Chemotherapy	15/20	7/7	22/27	9/12	1/1	10/13
Doublet Chemotherapy	8/8	2/3	11/11	6/6	0/0	6/6
Overall Response	**49/57**	**11/12**	**60/69**	**18/21**	**1/1**	**19/22**
Prediction Accuracy %	**86 %**	**92 %**	**87 %**	**86 %**	**100 %**	**86 %**

Sens = sensitive ; Resis = Resistant

DRIT Prediction Accuracy

Breast Cancer

Hormonal Treatment

Treatment N=34	# Patients	DRIT PA	SOC PA	IHC PA
Aromatase Inhibitors	21	19/21	17/21	14/21
Tamoxifen	9	8/9	7/9	6/9
Faslodex	4	4/4	4/4	3/4
Total	**34**	**31/34**	**28/34**	**23/34**
% Response		**91.2 %**	**82.4 %**	**67.6 %**

PA= Predictive Accuracy; IHC = Immunohistochemistry; SOC = standard of care

DRIT Prediction Accuracy

Breast Cancer

Monochemotherapy/Trastuzumab

Treatment N=40	DRI	Prediction Accuracy (PA)		
		Sensitive Tumor	Resistant Tumor	Overall
Taxanes	Beta-Tubulin III	16/20	1/1	17/21
Capectabine	Thymidylate Synthase	7/11	3/3	10/14
Trastuzumab	HER-2/neu	1/1	4/4	5/5
Overall Response		**24/32**	**8/8**	**32/40**
% PA		**75 %**	**100 %**	**80 %**

Overall Results
Breast Cancer
DRIT vs Standard Care

Standard of Care (SOC) Treatment Success(TRS)					DRIT Prediction Accuracy (PA)			
Treatment	**Resp**	**Non-Resp**	**Obj. Resp**	**% TRS**	**Sens**	**Resis**	**Predicted Response**	**PA %**
Hormonal	28	6	28/34	82 %	29/32	2/2	31/34	90 %
Mono-Chemotherapy	24	16	24/40	56 %	24/32	8/8	32/40	82 %
Doublet Chemotherapy	14	3	14/17	73 %	14/14	2/3	16/17	91 %
Overall Response	**66**	**25**	**66/91**	**73 %**	**67/78**	**12/13**	**79/91**	**87 %**

DRIT Diagnostic Performance
Breast Cancer

N=91	Hormonal	Mono-Chemotherapy	Doublet Chemotherapy	Overall
Treatment interventions	34	40	17	91
Sensitivity	0.99	1	0.93	0.99
Specificity	0.4	0.50	1	0.52
Positive Predictive Value	0.86	0.74	1	0.86
Negative Predictive Value	0.92	1	0.67	0.92
Overall Accuracy	**0.90**	**0.80**	**0.94**	**0.87**

Conclusions

- DRIT measurement of estrogen receptor displays higher prediction accuracy than FDA approved commercial test
- DRIT displays a high degree of accuracy in predicting the response to non-hormonal therapies (chemotherapy & trastuzumab)
- DRIT predictions of treatment outcome outperformed the treatment success of the standard of care
- DRIT was able to identify ineffective treatments
- Preliminary results indicate that DRIT is applicable to a diverse population of patients

Drug Response Indicators

GI Cancer

- Drugs - FDA approved, NCCN recommended

DRUG	DRI
Anthracycline	Topoisomerase II
Irinotecan	Topoisomerase I
Taxanes/ Vinca Alkaloids	B-TUB III
5 FU	Thymidylate Synthase
Gemcitibine	Ribonucleotide Reductase
Platinum Salts	ERCC1

DRIT Performance Accuracy
GI Cancer

Treatment Category	Sensitive	Resistant	Predicted Response	% Prediction Accuracy
Mono-chemotherapy	10/12	1/1	11/13	86.7 %
Doublet Chemotherapy	21/25	0/0	21/25	82.6 %
Triplet Chemotherapy	1/1	1/1	2/2	100 %
Overall Accuracy	**32/38**	**2/2**	**34/40**	**85.0 %**

DRIT Diagnostic Performance
GI Cancer

N=40	Mono-Chemotherapy	Doublet Chemotherapy	Triplet Chemotherapy	Overall
Treatment interventions	13	25	2	40
Sensitivity	1	1	1	1
Specificity	0.33	N/A	1	0.25
Positive Predictive Value	0.83	0.84	1	0.84
Negative Predictive Value	1	N/A	1	1
Overall Accuracy	0.87	0.83	1	0.85

Drug Response Indicator Test

Benefits for Patients

DRIT as a Diagnostic Service will:

- Individualize Anticancer Therapy Selection
- Lessen Exposure to Ineffective Anticancer Therapies
- Reduce the Need for Drug Combination Therapy
- Reduce Potential Side Effects to Patients
- Potential for Saving Significant Treatment Costs
- Improve Quality of Life

Drug Response Indicator Test
Cost Savings

- Based on Breast Cancer Retrospective Trials

Treatment	Resistant Tumors	Cost Savings
Endocrine ($7800)	2	$ 15,600
Taxane ($12,000)	1	$ 12,000
Capecitabine ($ 15,000)	3	$ 45,000
Herceptin ($50,000)	4	$ 200,000
Doublet Gemzar ($20,000) Taxane / Herceptin ($112,000)	2	$ 132,000
Overall Savings (Drug Costs)	12	$ 404,600
Ancillary Costs ($4,000/3 cycles)		$ 144,000
Total Savings		$ 548,600
Savings/patient		$ 8,000

Drug Response Indicator Test
Cost Savings – Single Drug

- Saving Example based on single Drug = Taxotere
- Assumption = 50 % efficacy
- Drug Cost/ 100 patients = $ 1,757,700
- Drug Savings / resistant patients = $ 878,850
- Per patient Savings = $ 8,788
- Savings/ US annual breast cancer population (40,000 patients)= $ 351M

Drug Response Indicator Test

Competitive Advantage

- Rigorous in vitro qualification of all Drug Response Indicators
- Direct imaging of a specified target within a tissue sample
- Application to tissue sections prepared from paraffin blocks
- Standardization of fluorescent measurement
- Adaptable to new chemotherapeutic drugs
- Adaptable to additional cancers and biomarkers
- Predicts tumor response for up to six (6) FDA-Approved Drugs