The I-SPY 2 trial in the US
the role of biomarkers for treatment assignment

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UCSF Comprehensive Cancer Center, San Francisco
Netherlands Cancer Institute, Amsterdam
ISPY-2 Participating Organizations

Sponsor:
NCI, FDA, Pharma unrestricted

Investigational Agent Providers
Agents Approved and Under Consideration

Biomarker Device Providers
Stratifying & Qualifying

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<table>
<thead>
<tr>
<th>Sponsor: NCI, FDA, Pharma unrestricted</th>
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<th>Investigational Agent Providers</th>
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<tr>
<td>Abbott</td>
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<td>AMGEN</td>
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<td>Genentech</td>
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<td>MERCK</td>
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<td>Takeda</td>
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<td>Millennium</td>
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<td>Pfizer</td>
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<td>Wyeth</td>
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<tr>
<th>Biomarker Device Providers</th>
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<tbody>
<tr>
<td>BERKELEY LAB</td>
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<tr>
<td>MEDICAL</td>
</tr>
<tr>
<td>George Mason University</td>
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<tr>
<td>THERANOSTICS HEALTH</td>
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<tr>
<td>agendia™</td>
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Drug Development – Current Model

One FDA-Approved Drug - Start to Finish

- 10-15 Years
- 1,000 – 6,000 Volunteers
- $1 Billion
More Efficient Clinical Trial Process

Inefficient clinical trials account for a majority of the time and cost associated with the failures of the current system

- Reduce time to conclusive results/Accelerate learning
- Reduce patient's/volunteers required
- Reduce cost of conducting trials
- Increase collaboration/Data sharing
The “Neoadjuvant” Approach Dramatically Accelerates Knowledge Turns

Metastatic Approach: 2 to 4 year knowledge turn
Adjuvant Approach: 6 to 9 year knowledge turn

Neoadjuvant Approach: 1 year knowledge turn
I-SPY Trial Program

Investigation of Serial studies to Predict Your Therapeutic Response with Imaging and Molecular Analysis
Who benefits from what systemic therapy

• Therapy response prediction

I-SPY 2 neoadjuvant trial program

I-SPY PIs: Laura Esserman (UCSF)
         Don Berry (MDAnderson)
Trial Operations: Angie DeMichele (UPenn)
Drug selection: Doug Yee (UMinnesota)
Patient Advocates: Jne Perlmutter (AnnArbor)
Imaging: Nola Hylton (UCSF)
Biomarkers: Laura van ‘t Veer (UCSF)

Molecular Biomarkers: Chuck Perou, Angie DeMichele,
Marc Lenburg, Sarah Davis, Meredith Buxton, Chad Livasy,
Chip Petricoin, Denise Wolf, Joe Gray et al
I-SPY 1 Clinical Trial Backbone
CALGB 150007 / ACRIN 6657

Layered Imaging and Molecular Biomarker Studies Onto Standard Clinical Care Neo-adjuvant therapy

- Anthracycline
- Taxane
- Surgery & RT
- Tam if ER+

- Serial MRI Scans
- Serial Core Biopsies
Questions

• Does early response help us to predict early relapse?
  – Complete Pathologic Response: pCR
  – Residual Cancer Burden: RCB

• How do the molecular signatures impact on the interpretation of pCR and RCB?
Trial Endpoints

- **Early**
  - MRI response after 1 cycle of chemotherapy
    - Longest Diameter, Volume

- **Intermediate**
  - pCR Pathologic Complete Response
  - RCB Residual Cancer Burden
  - % change in MR volume

- **Late**
  - 3 year Recurrence Free Survival
  - 3 year Overall Survival
Response measure at time of surgery: Residual Cancer Burden

• Integrates several pathologic features
  – Lymph node status
  – Extent of Tumor Bed
  – Tumor size
  – Tumor cellularity

• Output is continuous or 4 discrete categories
  – RCB 0 pCR, no invasive tumor
  – RCB I scattered residual disease
  – RCB II moderate tumor burden
  – RCB III significant tumor burden

Symmans et al JCO 2007
<table>
<thead>
<tr>
<th>Institution Name</th>
<th>Accrual</th>
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<tr>
<td>University of Pennsylvania Medical Center</td>
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<tr>
<td>Georgetown University Hospital</td>
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<tr>
<td>University of North Carolina</td>
<td>36</td>
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<tr>
<td>Memorial Sloan Kettering Cancer Center</td>
<td>22</td>
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<tr>
<td>University of Washington</td>
<td>5</td>
</tr>
<tr>
<td>University of Alabama at Birmingham Medical Center</td>
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<td>University of Chicago</td>
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<tr>
<td>University of Texas Southwestern</td>
<td>14</td>
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<tr>
<td>University of California San Francisco</td>
<td>66</td>
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</table>

- 1042 frozen cores from 201 patients
- 1301 paraffin cores from 223 patients
- 948 serum samples from 158 patients.
Patients Accrued
n=237

Patients Withdrawn
n=16

Patients who didn’t have surgery
n=6

Patients with pathology assessment after Neoadjuvant Therapy
n=215

Patients without RCB
n=14

Patients with pCR and RCB
n=201
Questions

• Does early response help us to predict early relapse?
  – Complete Pathologic Response: pCR
  – Residual Cancer Burden: RCB

• How do the molecular signatures impact on the interpretation of pCR and RCB?
Relationship of pCR and RCB with Early Relapse for all I-SPY 1 Patients

Relapse-free Proportion

Years since surgery

Relapse-free Proportion

Years since surgery

pCR (n=58)

No pCR (n=157)

RCB 0 (n=56)
RCB I (n=18)
RCB II (n=86)
RCB III (n=41)
Questions

• Does early response help us to predict early relapse?
  – Complete Pathologic Response: pCR
  – Residual Cancer Burden: RCB

• How do the molecular signatures impact on the interpretation of pCR and RCB?
I-SPY 1 Biomarker Platforms

Tissue: Core

H&E, IHC, FISH  Expression Arrays  p53 GeneChip  Protein Arrays (RPMA)

UNC, Penn  UNC, UCSF, NKI  UNC  GMU

CGH

Id1 proteins  autoantibodies  phospho proteins

Serum  UCSF
Tissue Distribution & Analyses Schema

Sample

Paraffin

Check for Tumor Presence

Initial H&E

UNC: Dressler Lab

PENN: DeMichelle Lab

Her2 Protein Over expression

Her2, TopoII Amplification

IHC

FISH

2 Frozen Cores

UCSF

Check for Tumor Presence

Initial H&E

Proteomics

GMU: Liotta/Petricoin Lab

Tumor Enriched

30%

UNC: Perou Lab

NKI: vantVeer Lab

Gene Expression

RNA

UCSF: Gray Lab

Gene Chip For P53

RNA

70%

DNA

MDACC: Pusztai/Symmans Lab

Storage

1 Frozen Core

Storage

Core Remainder

Her2 Protein Over expression

Her2, TopoII Amplification

Data uploaded in

NCI calIntegrator

NCI: caBIG, Madhavan

UCSC Cancer Genomics Browser

UCSC: Haussler, Kent, Zhu, Wang

What’s been done 1/09:

- 44K Agilent gene expression array data
- cDNA microarray
- MIP (CGH) array
- p53 sequencing
- RPMA
- IHC/FISH
I-SPY: Majority Poor Prognosis Tumors

NKI 70 Gene Profile

“Good” Signature  9%
“Poor” Signature  91%

Mean Tumor Size = 6.0
Present as clinical mass
55% < Age 50
## pCR Rates: RNA Classifiers

<table>
<thead>
<tr>
<th>Gene Profile</th>
<th>Distribution (n = 149)</th>
<th>pCR (n = 144)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ROR-S (intr subtypes)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>26%</td>
<td>5%</td>
<td>8.8 x 10^-4</td>
</tr>
<tr>
<td>Moderate</td>
<td>38%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>37%</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td><strong>NKI 70</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good Signature</td>
<td>9%</td>
<td>0%</td>
<td>0.038</td>
</tr>
<tr>
<td>Poor Signature</td>
<td>91%</td>
<td>27%</td>
<td></td>
</tr>
<tr>
<td><strong>Wound Healing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quiescent</td>
<td>23%</td>
<td>6%</td>
<td>0.0049</td>
</tr>
<tr>
<td>Activated</td>
<td>77%</td>
<td>30%</td>
<td></td>
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<tr>
<td><strong>p53 Mutation Gene signature</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Wildtype</td>
<td>50%</td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td>Mutation</td>
<td>50%</td>
<td>38%</td>
<td>3.7 x 10^-4</td>
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Relationship of RCB with Early Relapse for ‘poor biology’ I-SPY 1 Patients

- Good response = RCB 0 and I
- Poor response = RCB III

Log-rank $P = 5.5 \times 10^{-7}$
Recurrence-free survival after neoadjuvant therapy:
1) Good Prognosis Biology Tumors

All do well REGARDLESS of pathological response (pCR and non-pCR) in neo-adjuvant phase

No response, still good outcome, risk of recurrence low

Good Biology Tumors do not benefit from Chemotherapy

Esserman et al, DeMichele et al, van’t Veer et al, ASCO, ASCO Breast, SABCS 2009
Recurrence-free survival after neoadjuvant therapy:  
2) Poor Prognosis Biology Tumors

pCR (and RCB) in neo-adjuvant phase are VERY significant predictors of early relapse in the context of a poor prognosis profile

No response, no good outcome, risk of recurrence high

Response, better outcome, risk of recurrence lower

Poor Biology Tumors (subset) do benefit from Chemo

Esserman et al, DeMichele et al, van’t Veer et al, ASCO, ASCOBreast, SABCS 2009
Rapidly Learn to Tailor Agents
I-SPY 2
Adaptive Design, Integration of Biomarkers
I-SPY 2 is Designed to

- Screen phase 2 agents in combination with standard chemotherapy in neo-adjuvant setting
  - Endpoint is pCR
  - “threshold” for ‘graduation’ is 85% predicted likelihood of success in a 300-patient phase 3 trial for drug biomarker pair

- Select high risk biology patients only, in highest need of (more) effective therapies

- Accelerate process of identifying drugs that are effective for specific breast cancer subtypes
  - Integration of biomarkers

- Reduce the cost, time, and numbers of patients needed to get effective drugs to market
I-SPY 2 Adaptive Trial Outline

Accrual: Anticipate 800 patients over 3–4 years
Enroll: ~20 patients per month
Participating Sites: 15–20 across US and Canada
I-SPY 2 Adaptive Trial Schema: Screening & Randomization

Eligibility Assessment Process

Patient presents with newly diagnosed \( \geq 2.5 \text{cm} \) invasive tumor

Core biopsy to assess eligibility

Eligibility determined by:
- Ability to tolerate MRI
- Ability to generate 44k Agilent microarray

Is patient:
- **MammaPrint Low**
- **ER + and HER2 -**

Yes

No

Patient On Study
Randomized to treatment arm based on:
- ER, PR status
- HER2 Status
- MammaPrint score

Patient not on study
Not considered good candidate for chemotherapy

Patient On Study
# TARGET PATIENT POPULATIONS FOR PROPOSED TIER 1 AGENTS

<table>
<thead>
<tr>
<th>Agent</th>
<th>HER2+ / Any HR Cancers</th>
<th>HER2− / HR+ Cancers</th>
<th>HER2− / HR− Cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP Inhibitor</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>IGFR Inhibitor</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>HER2 TKI Inhibitor</td>
<td>Yes*</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>APO/TRAIL</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Vascular Disrupting Agent</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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*Investigational agent will be given in place of trastuzumab for HER2+ study participant.
I-SPY 2 Adaptive Trial:
Introduce several new agents for a given profile

Key
MRI
Residual Disease (Pathology)

HER 2 (+) Randomize
Taxol + Trastuzumab
Taxol + Trastuzumab + New Agent A
Taxol + Trastuzumab* + New Agent B
Taxol + Trastuzumab* + New Agent C

HER 2 (-) Randomize
Taxol
Taxol + New Agent C
Taxol + New Agent D
Taxol + New Agent E

AC
Surgery
Learn and adapt from each patient as we go along

*Or equivalent

Patient is on Study

MRI Residual Disease (Pathology)
I-SPY 2 Adaptive Trial: Learn, Drop, Graduate, and Replace Agents Over Time

**HER 2 (+)**
- Taxol + Trastuzumab
- Taxol + Trastuzumab + New Agent A
- Taxol + Trastuzumab + New Agent B
- Taxol + Trastuzumab + New Agent F

**HER 2 (−)**
- Taxol
- Taxol + New Agent F
- Taxol + New Agent GH
- Taxol + New Agent E

**Randomize**

**Surgery**
Learn and adapt from each patient as we go along

**Key**
- MRI
- Residual Disease (Pathology)

*Or equivalent*
First part - ‘Learning’
random randomization and observation

At start of trial:
patients randomly assigned to arm

all experimental arms
plus standard chemo
**First part - ‘Learning’**

**random randomization and observation**

At start of trial: patients randomly assigned to arm

At entry of trial: patients tumor biology assessed, ER, PR, Her2, MammaPrint-index (stratified per arm)

- All experimental arms plus standard chemo

- Type 1: e.g. Triple negative
- Type 2: e.g. ER pos MammaPrint-very high
- Type 3: e.g. ER pos
- Type 8: etc etc
First part - ‘Learning’
random randomization and observation

At start of trial: patients randomly assigned to arm
At entry of trial: patients tumor biology assessed, ER,PR,Her2, MammaPrint-index (stratified per arm)
At surgery: tumor response assessed (pCR=X) and evaluated for biology specific association

All experimental arms plus standard chemo

Type 1
Response drug 2
Type 2
Response drug 1
Type 3
Continued in to - ‘Adaptive’ part assigned randomization and evaluation

At entry of trial: assigned randomization based on patients tumor biology, ER, PR, Her2, MammaPrint-index

Biology type 2 -> drug 1 or control
Biology type 1 -> drug 2 or control

all experimental arms plus standard chemo
Continued in to - ‘Adaptive’ part
assigned randomization and evaluation

At entry of trial: assigned randomization based on patients tumor biology, ER, PR, Her2, MammaPrint-index
- Biology type 2: assigned to drug 1 or control
- Biology type 1: assigned to drug 2 or control

At surgery: tumor response assessed (pCR=X) and evaluated for biology specific association
- Endpoint is pCR
- “Threshold” is 85% predicted likelihood of success in a 300-patient phase 3 trial for drug biomarker pair
- Anticipated 100-120 patients needed per arm to find successful drug-biomarker combination or a failure
Biomarkers in I-SPY 2

- When a drug leaves the trial, we learn the probability of success to predict response for
  - Established/Approved Biomarkers
  - IDE Biomarkers
  - Qualifying Biomarkers
  - Exploratory Biomarkers (discovery of new markers of response prediction)
Qualifying Biomarker
Lawrence Berkeley National Lab 60 Cell Line Analysis

**Trial Preparation**

I-SPY 2 investigational agents are applied to the 60 LBNL Breast Cancer Cell Lines identified using the Panomics QuantiGene Plex 2.0 Assay.

Cell lines are evaluated based on response to agents to predict effectiveness of the agents by cell line.

**Participant Treatment**

Biopsy is taken from the trial participant’s tumor and predictive gene expression profile generated using the Panomics QuantiGene Plex 2.0 Assay in a CLIA certified lab.

Trial Participants are treated with an investigational agent based on trial randomization.

**Post-Treatment Analysis**

Actual participant responses are compared to predicted responses based on cell line signature.

Results of treatment on participants are evaluated.
Qualifying Biomarker: Predictive Markers
Lawrence Berkeley National Lab 60 Cell Line Analysis using the Panomics QuantiGene Plex 2.0 Assay

The participant’s tumor is matched to one of the 60 cell lines using the gene expression profile determined using the Panomics QuantiGene Plex 2.0 Assay.

Panomics QuantiGene Plex 2.0 Assay Work Flow

Step 1: Release Target RNA  Step 2: Target RNA Capture  Step 3: Signal Amplification  Step 4: Detection
Targeting MEK in 46 cell lines
Gray Lab – a pilot

Red: basal-type; Green: luminal-type cell lines

SABCS poster #2042 Wolf et al
Analysis of *in vitro* data using adaptive splines identified 406 genes predictive of response to CI1040, 135 and 271 were expressed *more highly in* CI1040-resistant or –sensitive cell lines respectively.
**in vitro** derived MEK response markers

Co-expressed predictor genes in cell lines also co-expressed in human tumor biopsies

Hierarchical clustering with 135 and 271 that were expressed more highly in CI1040-resistant or –sensitive cell lines respectively
I-SPY1 patient biopsies evaluated for MEK response markers

Hypothesis:

SABCS poster #2042
Wolf et al

Hypothesis: MEK inhibitor sensitive patients could potentially benefit

I-SPY clinical trial patients received standard taxol/anthracyclin neo-adjuvant therapy; biopsies pre-treatment analysed for gene expression
Median survival 3.6 years
Breast Cancer subtypes and marker identification to guide therapy

testing *in vitro* derived response markers in human breast cancer biopsies

• Existence of cell line response expression patterns in human tumors (Clinical trial I-SPY1)

• Provide a system were cell line response markers are ‘qualified’ in patients treated with the same drug (Clinical trial I-SPY2)

• Provide a system were validated markers can be used to drive treatment selection for specific drugs (Clinical trial I-SPY2)
neo-adjuvant design
integrating molecular and imaging data
to optimize effective treatment assignment