I-SPY 2: An Adaptive Breast Cancer Trial Design in the Setting of Neoadjuvant Chemotherapy

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I-SPY 2 (investigation of serial studies to predict your therapeutic response with imaging and molecular analysis 2) is a process targeting the rapid, focused clinical development of paired oncologic therapies and biomarkers. The framework is an adaptive phase II clinical trial design in the neoadjuvant setting for women with locally advanced breast cancer. I-SPY 2 is a collaborative effort among academic investigators, the National Cancer Institute, the US Food and Drug Administration, and the pharmaceutical and biotechnology industries under the auspices of the Foundation for the National Institutes of Health Biomarkers Consortium.

I-SPY 2 RATIONALE AND BACKGROUND

The daunting statistics that currently define cancer incidence and mortality require innovative strategies that will address the prohibitive expenditures of time and cost associated with the development of new oncology drugs. Although there are many promising new oncology drugs in the pipeline, the current process for development and regulatory review is inefficient and expensive, requiring a decade or more to complete. While biomarkers show promise for informing all aspects of oncology drug development, diagnosis, and treatment, clinical validation (qualification) has proved extremely difficult. The Cancer Steering Committee of the Foundation for the National Institutes of Health Biomarkers Consortium is taking several innovative approaches to remove this "biomarker barrier" in order to qualify both biomarkers and drugs for evidence-based development in clinical trials.

Over the past 20 years, significant progress has occurred in the detection and treatment of breast cancer. In fact, many women who present with stage I and II mammographically detected disease have excellent outcomes because of improved adjuvant therapy and lower risk of recurrence. Despite this progress, 10–15% of newly diagnosed breast cancers present as locally advanced cancers, with the likelihood of favorable long-term outcomes being significantly lower.[1] The absolute numbers of these cancers have not decreased over time, and successful treatment options remain limited. These patients continue to represent a disproportionately large fraction of those who die of their disease. Given that the standard of care for these women increasingly includes neoadjuvant therapy prior to surgical resection, this combination of group and setting represents a unique opportunity to learn how to tailor the treatment to patients with high-risk breast cancers.

Cancer research from the past decade has shown that breast cancer is a number of heterogeneous diseases; this finding suggests that directing drugs to molecular pathways that characterize the disease in subsets of patients will improve treatment efficacy. Currently, however, most phase II and III trials of new breast cancer drugs are in the metastatic setting, followed by randomized phase III registration trials in the adjuvant setting. These trials do not reflect the fact that there is a wide range of molecular characteristics of the patient's disease. Adjuvant trials require long-term follow-up and the enrollment of many thousands of patients,[2] and it may take 10–20 years[3] to gain marketing approval for successful drugs. Moreover, substantial investments of time and other resources are required for the development of drugs that ultimately fail. Although the use of biomarkers (molecular profiles, protein pathways, imaging, etc.) in the selection of patient populations for tailored studies of new drugs is promising, developing translational approaches in clinical trials for prediction of drug response presents a major challenge. The development and use of biomarkers for early measures of therapeutic response would facilitate the efficient evaluation of new agents in focused early clinical trials[4] and enable the development of more informed, smaller phase III trials.

I-SPY 2 represents a unique approach toward addressing the "biomarker barrier." It will be performed as a neoadjuvant trial in women with large primary cancers of the breast (>3.0 cm), and the end point for response to treatment will be the measurement of pathologic complete response. I-SPY 2 will also test, analytically validate, and qualify biomarkers as new drugs are tested; employ an adaptive trial design to enable efficient learning

about each drug's biomarker signature; and utilize organizational management principles and sophisticated bioinformatics in order to eliminate the current inefficiencies in clinical trials.

I-SPY 2 evolved from a previous program, I-SPY 1. I-SPY 1 was a collaboration of the National Cancer Institute Specialized Programs of Research Excellence, the American College of Radiology Imaging Network; the Cancer and Leukemia Group B; and the National Cancer Institute Center for Biomedical Informatics and Information Technology. This first trial was designed to connect clinical, laboratory, and bioinformatics investigators with a new model for the evaluation of neoadjuvant chemotherapy in the setting of locally advanced breast cancer: bringing together data from multiple molecular biomarker studies with imaging. In I-SPY 1, chemotherapy was administered prior to surgery, and test biomarkers were compared with tumor response on the basis of magnetic resonance imaging (MRI), pathologic residual disease at the time of surgical excision, and 3-year disease-free survival. I-SPY 1 demonstrated that a collaborating group of investigators could effectively integrate biomarkers and imaging into the course of care by agreeing on standards for data collection, biomarker assessment, and MRI. The group also developed and shared methods to optimize assays, small amounts of frozen core biopsy material, tools for tissue tracking, and common information management platforms and repositories.[5–9] This robust infrastructure will be leveraged to support I-SPY 2.

I-SPY 2 TRIAL DESIGN

I-SPY 2 will compare the efficacy of novel drugs in combination with standard chemotherapy with the efficacy of standard therapy alone. The goal is to identify improved treatment regimens for patient subsets on the basis of molecular characteristics (biomarker signatures) of their disease. As described for previous adaptive trials,[10] regimens that show a high Bayesian predictive probability of being more effective than standard therapy will graduate from the trial with their corresponding biomarker signature(s). Regimens will be dropped if they show a low probability of improved efficacy with any biomarker signature.

New drugs will enter as those that have undergone testing are graduated or dropped.

Biomarkers

Biomarkers for I-SPY 2 will consist of three distinct classes. *Standard biomarkers*, accepted and approved by the Food and Drug Administration, will be used to determine patient eligibility and randomization for the trial. *Qualifying biomarkers* will be those that are not yet approved by the Food and Drug Administration but show promise for determining patient eligibility or measuring treatment response; those with sufficient existing data will be evaluated under Investigational Device Exemptions, whereas those with less robust data will be tested in Clinical Laboratory Improvement Amendments–certified laboratories to further develop evidence needed for FDA approval. *Exploratory biomarkers* will be those that are of interest on the basis of promising preliminary data suggesting predictive or prognostic value for breast cancer treatment. Well-annotated tissue and blood samples collected prospectively in I-SPY 2 will contribute to the analytical validation and qualification of exploratory biomarkers, both during the trial and retrospectively.

Patient stratification

Standard biomarkers will be used to define the initial signatures against which treatment will be assigned: hormone receptor status (+/−), human epidermal growth factor receptor 2 (HER2) status (+/−), and MammaPrint[11,12] status (highest MP2, other MP1). **Figure 1** shows that estrogen receptor, progesterone receptor, and HER2 status as assessed by community immunohistochemistry or fluorescence *in situ* hybridization will be part of the routine diagnostic workup for determining patient eligibility. Two additional assays of HER2 (qualifying biomarkers) will be performed during I-SPY 2.

Data from the qualifying biomarker class will be evaluated for their sensitivity and specificity for stratifying patients and/or for predicting pathologic complete response. Successful qualifying biomarkers will be used to improve randomization
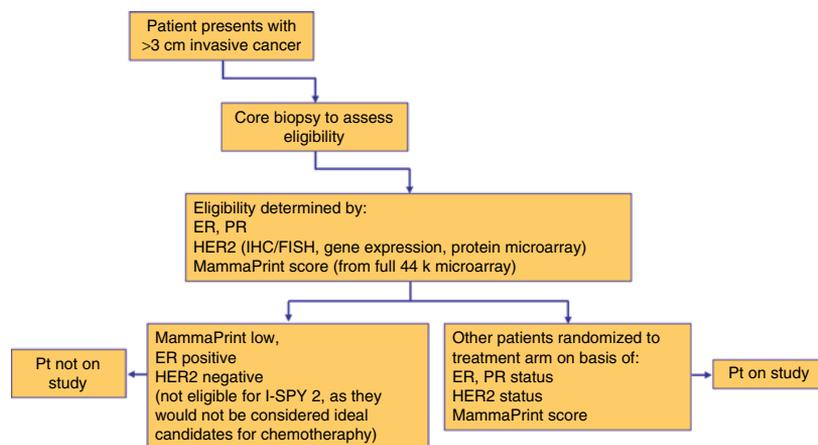
Figure 1 I-SPY 2 eligibility and treatment assignment. ER, estrogen receptor; FISH, fluorescence *in situ* hybridization; HER2, human epidermal growth factor receptor 2; I-SPY 2, investigation of serial studies to predict your therapeutic response with imaging and molecular analysis 2; IHC, immunohistochemistry; PR, progesterone receptor; Pt, patient. For MammaPrint scoring, see refs. 11,12.

and treatment as the trial progresses. For example, HER2 gene expression will be evaluated using the Agendia 44 k full genome microarray,[11,12] and phosphorylated HER2 (pHER2) will be assayed using reverse phase protein microarray.[13] Also, in view of the fact that I-SPY showed MRI volume to be the best predictor of residual disease after the administration of chemotherapy,[14,15] the measurement of MR volume at baseline and during and after treatment will be automated and used to inform the randomization of patients as the trial proceeds.

Overall clinical trial design

The overall trial design for I-SPY 2 (**Figure 2**) will feature two arms of a standard neoadjuvant chemotherapy regimen, starting with weekly paclitaxel (plus trastuzumab (Herceptin) for HER2+ patients) followed by doxorubicin (Adriamycin) and cyclophosphamide (Cytoxan). In the other arms, five new drugs will be tested simultaneously, each being added to standard therapy. On the basis of statistical models, each drug will be tested in a minimum of 20 patients and a maximum of 120 patients. Following an initial core biopsy, MRI and blood sample draw to determine biomarker signature and eligibility (**Figure 1**), patients will be randomized to the novel drug agents, which will be administered weekly during the paclitaxel phase of the trial. After 3 weeks of the assigned treatment, patients will undergo a repeat MRI and core biopsy and continue treatment for 9 additional weeks. A third MRI and core biopsy will be performed prior to initiating standard chemotherapy with doxorubicin and cyclophosphamide, and a blood sample draw as well as a fourth MRI will be performed prior to surgery. Tumor tissue will be collected at surgery to assess whether the patient has pathologic complete response. This is the primary trial end point, but patients will also be followed for disease-free and overall survival for up to 10 years.

Adaptive statistical design

Drugs will be evaluated against biomarker signatures consisting of combinations of hormone receptor + or −, HER2 + or −, and two levels of MammaPrint scores. Although this design produces 256 possible signatures, most are biologically uninteresting or represent only small markets. Fourteen signatures of possible

interest based on the biology they represent and their expected high prevalence in the study population have been characterized for I-SPY 2. Several of these signatures represent disease types for which there is a widely recognized need for improved treatment—for example, HER2+ tumors; hormone receptor and HER2− tumors (triple-negative disease); and tumors with poor prognosis on the basis of having the highest MammaPrint score level (**Supplementary Table S1** online). In order to obtain information about treatment effects as early as possible, relationships between pathologic complete response and baseline and longitudinal markers will be modeled, and outcomes will be assessed continually during the trial. Randomization probabilities will be determined using the accumulating data pertaining to all the drugs in the trial. The trial is designed to "learn" over time which profiles predict response to each drug.

For the assignment of drugs to patients, Bayesian methods of adaptive randomization[10] will be used to achieve a higher probability of efficacy. Drugs that do well within a specific molecular signature will be preferentially assigned within that signature and will progress through the trial more rapidly. Each drug's Bayesian predictive probability[10] of being successful in a phase III confirmatory trial will be calculated for each possible signature. Drugs will be dropped from the trial for reasons of futility when this probability drops sufficiently low for all signatures. Drugs will be graduated at an interim point, should this probability reach a sufficient level for one or more signatures. Drugs that have high Bayesian predictive probability of being more effective than standard therapy will graduate along with their corresponding biomarker signatures, allowing these agent–biomarker(s) combinations to be tested in smaller phase III trials. When the drug graduates, its predictive probability will be provided to the company for all the signatures tested. Depending on the patient accrual rate, new drugs can be added at any time during the trial as other drugs are either dropped or graduated.

Investigational drugs

In order to enter I-SPY 2, drugs must meet specific criteria relating to safety and efficacy (**Table 1**). A candidate drug is
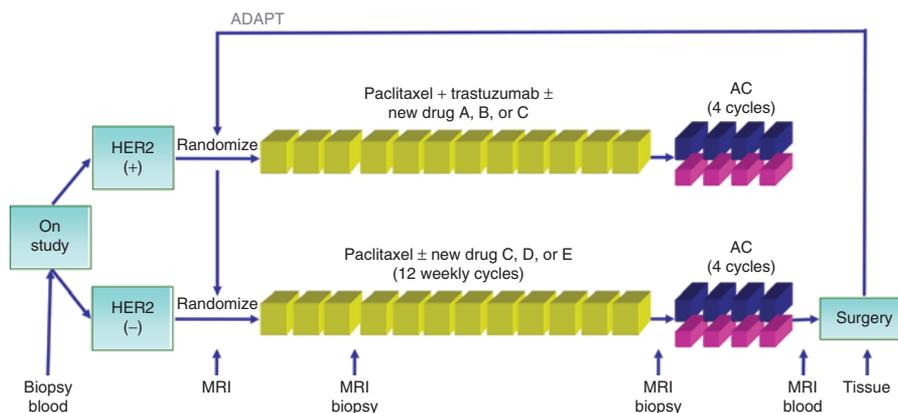
Figure 2 I-SPY 2 trial design. For HER2+ patients in the study, some new drugs with specific anti-HER2 activity may be administered in lieu of trastuzumab: anthracycline (AC) (e.g., doxorubicin) and cyclophosphamide (Cytoxan). HER2, human epidermal growth factor receptor 2; I-SPY 2, investigation of serial studies to predict your therapeutic response with imaging and molecular analysis 2; MRI, magnetic resonance imaging.

Table 1 Summary of criteria for I-SPY 2 drugs

Phase I testing completed
Compatible with standard taxane therapy (i.e., no unacceptable additive toxicity) and for HER2/*neu*–directed agents, compatibility with taxane plus trastuzumab therapy
Rationale or known efficacy in breast cancer
Fits strategic model for optimizing combinations of single/multiple molecular targeting drugs with or without standard chemotherapy
Targets key pathways/molecules in breast cancer: HER2 (Hsp90, HER2, HER3), IGFR, PI3K, macrophage, Akt, Akt + MAPK, PI3K + MEK, death receptor, cMET, mTOR + X
Sufficient availability of the drug

HER2, human epidermal growth factor receptor 2; I-SPY 2, investigation of serial studies to predict your therapeutic response with imaging and molecular analysis 2; IGFR, insulin-like growth factor receptor; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein/extracellular signal–regulated kinase kinase; mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol 3-kinase.

required to have been tested and found safe in at least one phase I clinical study with a taxane (or for HER2^{+} subjects, taxane plus trastuzumab), and there should be evidence of its potential efficacy against breast cancer from preclinical or clinical studies. Given that many companies produce a number of drugs with similar mechanisms of action and potential ranges of efficacy, the collaborators have agreed that testing representative drugs from a class will provide information to all partners about that class of drugs. This will allow all the companies to drop a drug from further consideration and to design additional phase II or focused phase III trials for their own drugs. Under special circumstances this may also be expanded to include more than one drug from a class. The pharmaceutical and biotechnology companies contributing drugs to I-SPY 2 are critical partners. Drugs are selected through a multitiered process that begins with the drawing up of a candidate list and is followed by in-depth discussions with interested companies. An independent group of experts makes the final selection on the basis of phase I safety data and preclinical and clinical data.

BIOINFORMATICS FOR COLLABORATION

A major advantage for I-SPY 2 is the sophisticated informatics portal initially developed for I-SPY 1. This infrastructure addresses the need to integrate and interpret enormous amounts of complex and disparate data (genomics, proteomics, pathology, and imaging) from many investigators, and it provides real-time access to study data for effective adaptation in the trial. This portal serves as a model for multidisciplinary collaboration and will be expanded for I-SPY 2 under the auspices of the Center for Biomedical Informatics and Information Technology.

SUMMARY

I-SPY 2, performed in the neoadjuvant setting, focuses on women with high-risk, locally advanced breast cancer identified at a stage when a cure is possible. The adaptive design approach provides a model for rapid assessment of novel phase II drugs and identification of effective drugs and drug combinations so as to determine which breast cancer subtypes will benefit. Specifically, learning will occur as the trial proceeds, and use of information from each patient will inform subsequent treatment assignments. Given the highly competent I-SPY 2 team, existing infrastructure from I-SPY 1, adaptive trial design, existing and developing biomarker candidates and test drugs, and the potential to learn what works within months rather than years, this initiative promises to be transformational for patients with breast cancer.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

CONFLICT OF INTEREST
The authors declared no conflict of interest.

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