New Omics Information for Clinical Trial Utility in the Primary Setting

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Cancer is a complex cellular disease caused by multiple factors via genetic mutations (hereditary or somatic) or environmental factors. The emerging omics technologies, including genomics, epigenomics, transcriptomics, proteomics, metabolomics, and interactomics, are increasingly being used for cancer research and personalized medicine; they have provided new opportunities in the molecular analysis of human cancer with unprecedented speed and detail. The omic approach has brought powerful ability to screen cancer cells at different levels from gene to metabolite and to search for novel drug targets, expounding the drug mechanism of action, identifying adverse effects in unexpected interaction, validating current drug targets, exploring potential applications for novel drugs, and enabling the translation from bench to bedside. As a clinical research tool, the neoadjuvant approach in breast cancer is the perfect setting for individualization of treatment based on clinical, pathological, image-guided, or molecular assessment, based on the omics techniques of tumors during treatment; neoadjuvant treatment offers the ability to discern treatment effect in vivo and may allow smaller trials targeting specific breast cancer subtypes.

Personalized medicine is an emerging field that promises to bring radical changes in health care. It can be defined as "a medical model that uses molecular profiling technologies for tailoring the right therapeutic option for the right person at the right time and being potentially able to determine the predisposition to disease at the population level and to deliver timely and stratified prevention" (1–3). The sequencing of the human genome together with the development and implementation of new high-throughput technologies (so-called omics) have greatly enhanced not only our knowledge on human cancer biology but have also opened up the ways to breakthroughs bringing much closer the possibility to a personalized patient treatment. Although all these omics have been widely used at experimental level to identify new targets, to define the impact of alteration on activity of classical anticancer agents or target-oriented drugs, and to characterize the toxicological profile of new drugs leading to a dramatic improvement in our knowledge on the molecular basis of cancer, we are still far from having clarified the entire picture. There is therefore the need to support studies aimed at defining the mechanisms of cancer biology as this will lead to the rational discovery of omics-based markers and the development of more reliable preclinical disease models. Several omics platforms, particularly those for the analysis of nucleic acids, are ready for clinical application but others, such as proteomics, epigenomics, cell functional analysis, etc, require further development before they can contribute to large-scale clinical translational studies that aim at identifying and validating biomarkers and biosignatures. Breast cancer represents the oncologic field in which application of these omics has been most widely applied. Several translational clinical trials have introduced genomic and transcriptomic studies with the aim of selecting patients more likely to respond to a given therapy (4–7). For example, it has been suggested that gene expression signatures of breast carcinomas may not only lead to new classifications of tumor subgroups but also carry prognostic and predictive information. Gene profiling studies have consistently showed that estrogen receptor (ER)–negative and ER-positive breast cancers are different biological diseases and that each of them can be further divided in subgroups with distinct disease-free and overall survivals and thus different oncological approaches are needed. This new molecular classification of breast cancer has been reproduced across different laboratories, patient populations, and array platforms (8).

We will first summarize the different omics technologies that have been developed and then discuss how these have been applied in the context of the primary setting of breast cancer.

Epigenomics

Epigenetic alterations are those alterations found in DNA not affecting its primary structure, which ends up with aberrant transcription. DNA methylation, remodeling, and histone modifications represent the different level of epigenetic alterations found in human cells all interacting one to each other. In breast cancer, several genes are found methylated, and a temptative signature of genes methylated has been found associated with prognosis (9,10). Epigenetic silencing in breast cancer is in fact not only associated with tumor initiation and progression but also in the development of drug resistance. Genome-wide methylation analysis allows the detection of whole methylation as well as specific gene methylation pattern in clinical specimen. The development of bisulfite conversion–based technique greatly facilitated the detection of methylated genes, and the combination with real-time polymerase chain reaction made possible a quantitation of gene methylation. Together with DNA

methylation analysis, histone modifications are gaining importance in epigenetics (11,12). Histone cores undergo a series of posttranslational modifications (methylation, acetylation, ubiquitination, sumoylation), which are important for chromatin structure. The analysis of histone modifications in clinical specimens is now possible with a relatively high-throughput analysis represented by Chromatin Immunoprecipition-on-chip and recently by Chip-SAGE (9).

Metabolomics

Metabolomics refers to the assessment of metabolic potential of an individual (through pharmacogenomic and detailed P450s analysis), and it is extremely useful in predicting toxicity of certain actively metabolized drugs, depending on the low- or high-metabolizing capacities of the patient. More challenging is the global metabolomic analysis, which is the detection of small molecules in organic fluids and tissues, often representing end product of cellular processes. The increasing availability of methods in nuclear magnetic resonance and mass spectrometry able to detect with high accuracy the levels of small molecules allows the detection of these end products, which reflect the sum of the response to a biological treatment or to the uncontrolled growth characteristics of the tumor (13–15). Metabolomics could be regarded as a potential way to classify tumors and to predict response to treatment. Even the metabolomic-based classification has been applied in breast cancer, no direct application in patient's selection in clinical trials has yet emerged (16–18).

Proteomics

Highly sensitive and specific techniques, which are able to detect low levels of proteins in virtually all clinical specimens, have been developed. Technologies such as matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry, surface-enhanced laser desorption/ionization-mass spectrometry, liquid chromatography-mass spectrometry, isotope-coded affinity tags, and isobaric tags for relative and absolute quantitation are now quite accessible to different laboratories, although the complexity of sample preparation and analysis remains high, relative to genomic and transcriptomic analysis (19). Nevertheless, proteomics analyses have been used to try to identify biomarkers for diagnosis and response assessment (20–24). Although in clinical trials in breast cancer, protein analysis (ie, ER, progesterone receptor, HER2) is used to stratify patients as luminal A or B. For HER2 subtype, triple negative (25), a more comprehensive proteomic analysis is warranted. Different kinases are expressed in different clinical subsets of breast cancer. Bianchini et al. (26) recently, using omics approaches assessed kinase expression patterns in different clinical subtypes of breast cancer, evaluated the prognostic and predictive values of kinase metagenes; they identified that kinases regulating mitosis and immune functions convey distinct prognostic information that varies by clinical subtype.

Indeed, the development of complex networks of protein interactions is likely to be more useful in the definition of new strategies in clinical trials, and in the near future, these techniques will be incorporated in clinical designs. In addition, the assessment of the functional status of a protein can be more informative, and this can be done analyzing the posttranslational protein modifications (such as phosphorylation, acetylation, and methylation). Furthermore,

the analysis of specific "subproteomic" blocks, like the analysis of all the secreted proteins, the secretome could be an additional tool to determine which signaling pathway is potentially driving for the tumor and increase the chance of a more selective therapy (27,28).

## Statistomics in Clinical Trials With Biological Targets

The Bayesian approach is an ideal statistical method in designing and analyzing clinical trials based on biological targets (29,30) as it allows: 1) the use of all the available evidence combining many sources of information (including "expert opinions") and borrowing strength across patients within the same trial and across trials; 2) the guarantee of a higher degree of flexibility; in a Bayesian trial, the sample size need not be chosen in advance, but the go and stop decision can be made at any time as more evidence becomes available; 3) for the provision of methods to calculate predictive probabilities regarding future events (like the probability that a certain subject will respond to a new therapy) that do not depend upon unknown parameters. These characteristics have implications for analysis as well as design. Typical goals in the Bayesian approach include faster learning via more effective designs of trials and more efficient drug development along with better treatment of patients who participate in the trial.

Omics in Primary Setting of Breast Cancer

The development of molecular profiling technologies to assess DNA, RNA, protein, and metabolites provides the potential to tailor medical care both at the tumor and patient levels. Indeed, the personalized approach offers the real opportunity to increase the therapeutic efficacy by targeting the genomic abnormalities driving tumors while decreasing the possible side effects of the therapy due to altered metabolism depending on the patients' genetic background (Figure 1). The development and use of Herceptin, designed to target HER2-positive tumors, is an excellent example of advances in tailoring breast cancer treatment to biology. In both the pivotal trastuzumab as single-agent and in combination with chemotherapy trials, responses were seen only in patients where the "target" +3 overexpression of the HER2 receptor protein detected by immunohistochemistry, or amplification of its gene detected by fluorescence in situ hybridization, was present (31–33).

When the activity of trastuzumab was analyzed in the entire breast cancer population, no major responses were seen. If based only on the results in this unselected population, an excellent drug would have been discarded as not worthy of further development.

The HER2 pathway story led the omics approaches to be largely used in breast cancer studies, reducing the distance between the laboratory and the clinical department, till merging them to put the discoveries coming from the bench to the bedside as a tool to a better understanding of tumor behavior. In the era of targeted therapy, breast oncologists started using "translation research" terminology more often in their approach to treatment (eg, gene profile, fluorescence in situ hybridization, mutations/sequencing, etc.) till individuating different steps of the translation research spectrum in their clinical practice (Table 1) as follows:
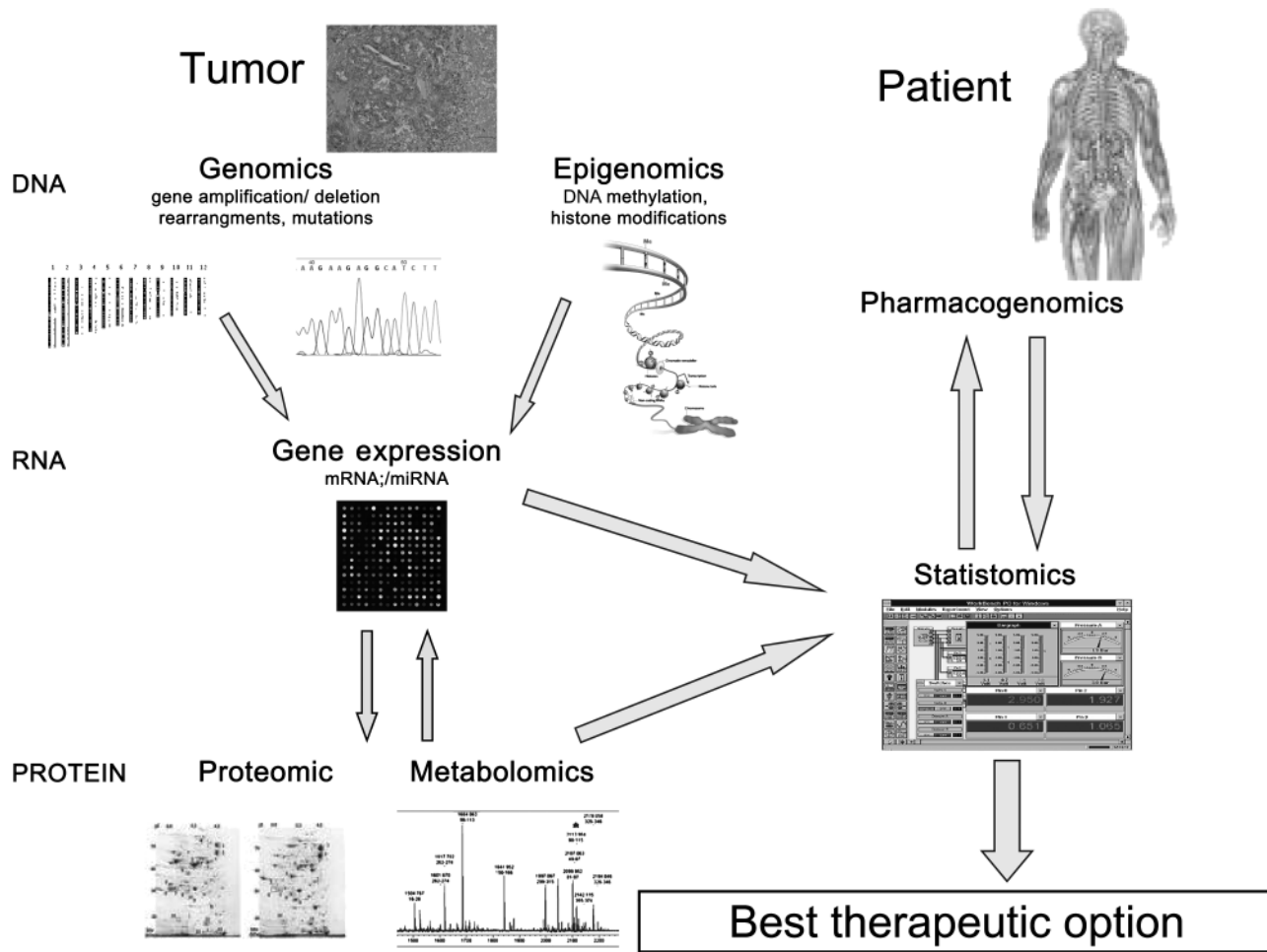
Figure 1. Schematic highthroughput approach for the selection the best therapeutic option for cancer patients. Ideally, the integrated approach will take into account the different aspects of tumor (at the DNA, RNA, and protein levels) and patient (pharmacogenomics), and, with the aid of statistic analysis tools, will generate an integrated tumor/patient signature specific for each patient.

- Step point 1: translation of basic research into clinical application.
- Step point 2: clinical application to evidence-based practice guidelines.

The two major applications of translational research are the identification of predictive factors and of new therapeutic targets. The majority of breast cancer patients are offered some type of systemic therapy. However, traditional anticancer therapy is empirical, and it extrapolates to an individual patient the conclusions of studies carried out in wide populations, with a high likelihood that many patients are treated to benefit a few. In general, neither side effects nor absolute benefit can be reliably predicted for an individual patient. Therefore, the main challenge for the oncology community has become the identification of predictive factors that will help select the optimal therapeutic strategy for each individual patient. This better selection of patients and treatment-tailoring is essential to avoid both overtreatment, with its potential for severe complications, and undertreatment with its deleterious consequences for patient survival.

In this context, the primary setting of breast cancer offers a unique emerging possibility for the both the rapid validation of biomarkers, as the anatomical accessibility of the breast provides the potential for serial biopsies to investigate molecular changes during treatment; as well as the possibility to identify new effective treatment strategies minimizing treatment-related side effects, to study the mechanism

Table 1. The step-points of the translation research in oncology

The translation research workflow in oncology			
Translation research spectrum		Setting	Examples
Step point 1	Discovery of new	Phase I and II clinical trial	HER2 and tumor aggressiveness; mechanisms of action—trastuzumab
Step point 2	Health application to evidence-based medicine	Phase III and IV clinical trial; observational studies	HER2 expression in breast cancer and trastuzumab/population benefits

of action of a drug, and to develop clinically applicable prognostic and predictive biomarkers in an attempt to individualize therapy.

Recently, a study based on the predictive role of MammaPrint in relation to pathological complete response after neoadjuvant treatment was published. The study shows that tumors with a poor 70-gene signature are more likely to achieve a pathological complete response (pCR), whereas tumors with a good prognosis signature are not. This finding has several important clinical implications: 1) a real application of genomics in a clinical setting; 2) the 70-gene profile could help in selecting patients for adjuvant chemotherapy, whereby chemotherapy is frequently withheld for tumors with a good prognosis signature (lower risk of relapse and tumor less sensitive to chemotherapy); 3) we also believe that the stratification of subjects according to the 70-gene profile could be helpful in controlled trials investigating the effectiveness of new drugs or new combinations of drugs.

In this setting, the opportunity to develop a more personalized treatment in an accelerated time frame is another great advantage. In fact, traditional drug development processes utilize adjuvant trials requiring long follow-ups, many thousands of patients, and many years to go from laboratory bench to patient bedside.

Table 2 reports a number of trials where omics have been applied. The I-SPY trial (Investigation of Serial Studies to Predict Your Therapeutic Response with Imaging And moLecular analysis) had the goal to identify indicators of response to neoadjuvant chemotherapy predicting survival in women with high-risk (stage II–III) breast cancer. This trial was designed to connect clinical, laboratory, and bioinformatics investigators with a novel model for the evaluation of neoadjuvant chemotherapy in locally advanced breast cancer, trying to bring together data from multiple molecular biomarkers with magnetic resonance imaging. In I-SPY 1, chemotherapy was administered before surgery and biomarkers were compared with tumor response assessed by magnetic resonance imaging, pathological residual disease at the time of surgery, and a 3-year disease-free survival. This trial demonstrates that a collaborating group of investigators could effectively integrate biomarkers and imaging by agreeing on standards for data collection, biomarker assessment, and magnetic resonance imaging. The robust infrastructure built up during I-SPY 1 was instrumental for the setting up of the ongoing I-SPY 2 trial launched in 2010 (I-SPY 2: An Adaptive Breast Cancer Trial Design in the Setting of Neoadjuvant Chemotherapy). This trial will use biomarkers to identify women who might benefit from investigational new drugs given along with standard neoadjuvant chemotherapy (chemotherapy given before surgery) with paclitaxel, doxorubicin, and cyclophosphamide. The trial will enroll women who have early-stage breast cancer and a high risk of recurrence, as determined by ER status, HER2 status, and the MammaPrint test. There are four key transformative concepts in the I-SPY 2 trial that deserve comment:

1) It has an adaptive trial design. The patients' assignment to a specific investigational drug will change/adapt through the trial. The information of how patients respond to their treatment is based on the biological profile of their tumor and will determine the assignment of subsequent patients with the same tumor profile to the available treatment (Bayesian methods of adaptive randomization).

2) It has early endpoints. Tumor tissue will be collected at surgery to assess whether the patient has pathological complete response. This is the primary trial endpoint, but patients will also be followed for disease-free and overall survival for up to 10 years.

3) It has rapid/simultaneous multiple drug evaluation. The trial will concurrently test three to five investigational drugs from different companies against a single control group.

4) The testing of the concept of personalized medicine is based on the molecular tools that have been developed in the last decade. Patients will be randomized to specific treatment arms based on the US Food and Drug Administration–approved biomarker tests that define the biological profile of the patients tumor (ER status, HER2 status, and the MammaPrint test). In addition, newer promising biomarker tests can potentially be collected and could be correlated with tumor response.

This study has been designed to move promising new drugs (up to 12) into phase III clinical trials more quickly and cost-effectively than traditional phase II studies. 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. The real possibility of learning what works within months rather than years makes the information available from this trial transformational for patients with breast cancer.

Conclusions

Proof-of-concept projects demonstrating the feasibility of bringing early returns from the omics revolution to the health care should be considered. Exploratory and high-risk projects that aim to develop novel omics technologies for application in the clinical setting for the discovery of biomarkers or signatures are to be encouraged. Indeed, the ultimate impact of omics technologies on improving the delivery of personalized medicine will depend on demonstrating its cost-effective utility in daily practice. The cost of generating large-scale prospective biobanks/trials with high-quality outcome data is enormous. The successful integration of omics-based personalized medicine into clinical practice does not only require the demonstration of its clinical utility and cost-effectiveness but also the development of processes so that the relevant information is available to the correct health practitioner at the appropriate time. In addition, and to further advance personalized medicine, the National Health System should promote evidence-based multidisciplinary training of omics researchers, clinician scientists, quantitative scientists, clinical care and pharmas, biotechs, and health-care policy makers with the aim to introduce validated and cost-effective omics technologies, including clinical bioinformatics and statistics, into health-care systems.

Finally, as a cautionary and provocative note, we should not forget that if the theory of the existence of the cancer stem cell is correct, we should rethink all our concepts of determining "peripheral" tumor characteristics and focus on finding the Achilles' heel of the tumor, the cancer stem cell, which needs to be targeted to successfully eradicate the tumor. In these times in

Table 2. Breast cancer trials in the neoadjuvant setting with omics application*

Type of trial	Decription	Findings	References
Molecular genomic	I-SPY: multicenter clinical trial designed to evaluate the impact of chemotherapy before surgery on patients with locally advanced breast cancer. Biological markers as predictors of pCR and survival were considered.	The molecular profiles of locally advanced breast cancer tumors predicted the response of the tumors to chemotherapy drugs given in advance of surgery.	http://ncicb.nci. nih.gov/tools/ translation_ research/ispy.
	I-SPY 2: it was designed to efficiently screen multiple novel drugs. It includes an "adaptive design" in which drugs are assessed over the course of months—rather than decades—and the information used in real time to direct the course of a trial. It also will test the qualification of biomarkers to help accelerate the path to the identification and availability of successful tailored treatment options for women with locally advanced breast cancer.	Ongoing	(4)
	Phase II/III randomized study of neoadjuvant therapy of exemestane vs letrozole vs anastrozole in postmenopausal women with ER-positive to determine whether patients who have a high Ki67 value (>10%) after 2 weeks of neoadjuvant aromatase inhibitors treatment have a higher than expected pathological response rate to neoadjuvant chemotherapy (20%) than would be typically observed for postmenopausal patients with unselected ER-positive rich tumors.	Ongoing The result of this study would potentially indicate that an early assessment of proliferation could be a useful approach to the identification of a chemotherapy-sensitive subgroup of ER-positive tumors.	(34)
	Molecular signatures of neoadjuvant endocrine therapy for breast cancer: Characteristics of response or intrinsic resistance. Untreated postmenopausal patients with ER-positive breast cancers were treated for 4 months in a neoadjuvant setting with the aromatase inhibitor exemestane alone or with tamoxifen.	Matched pre- and posttreatment tumor samples from the same patient were analyzed by gene expression profiling and were correlated with response to treatment. Genes associated with response to endocrine therapy were identified that may distinguish ER-positive hormone-responsive breast cancers from ER-positive tumors that exhibit intrinsic or de novo resistance.	
	Expression profiles differentiate between breast cancers clinically responsive or resistant to letrozole.	Expression profiles on high-density microarray chips were related to clinical responses assessed from tumor volume measurements after 3 months of treatment in 52 patients with breast cancer.	
	Evaluation of a 30-gene paclitaxel, fluorouracil, doxorubicin, and cyclophosphamide (T/FAC) chemotherapy response predictor in a multicenter randomized trial in breast cancer	The 30-gene predictor (DLDA-30) could identify patients with greater than average sensitivity to (T/FAC) chemotherapy.	(7)
Epigenomic	Identification of DNA methylation biomarker to discriminate patients with locally advanced breast cancer who may benefit from neoadjuvant doxorubicin treatment.	Methylation of two out 14 genes analyzed (ABCB1 and GSRP1) were associated with improved survival, whereas methylation of FOXC1 seemed to be a protective factor against tumor invasiveness	(35)
Proteomic	Identification of markers of response in neoadjuvant paclitaxel/radiation therapy by histology-directed matrix-assisted desorption/ionization mass spectrometry (MALDI-MS).	Identification of differentially expressed peptides in tumor biopsies from pCR and non-pCR (nonresponder) patients. Gene expression arrays were also used to identify differentially expressed genes.	(36)
	Evaluation of changes in serum protein levels during neoadjuvant chemotherapy in HER2-positive breast cancer.	Using an LC-MALDI-TOF/MS procedure changes in serum protein levels of patients treated with neoadjuvant chemotherapy were found, and these changes partially differ between patients highly sensitive to chemotherapy and patients less responsive.	(37)
	Protein expression changes after neoadjuvant use of aromatase inhbitors in primary breast cancer.	By a proteomic approach (mass spectrometry) changes in protein expression (both up- and down-regulation) protein were found after aromatase inhibitor. Among these proteins, heat shock protein 70 demonstrated the most significant positive correlation with the clinical response of the patients.	(38)

* ER = estrogen receptor; pCR = pathological complete response.

which we are moving to a comprehensive analysis of tumor complexity as a whole, we should perhaps have a look back to a more reductionistic system.

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