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PROSPECTIVE

The Genomic Landscape of Breast Cancer as a Therapeutic Roadmap

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Summary: The application of high-throughput techniques to profile DNA, RNA, and protein in breast cancer samples from hundreds of patients has profoundly increased our knowledge of the disease. The etiologic events that drive breast cancer are finally coming into focus and should be used to set priorities for clinical trials. In this Prospective, we summarize some of the headline conclusions from 6 recent breast cancer "omics profiling" articles in *Nature*, with an emphasis on the implications for systemic therapy. *Cancer Discov; 3(1); 27–34. ©2012 AACR.*

INTRODUCTION

Over the past 6 months, 4 articles have appeared in *Nature* that describe the application of massively parallel sequencing techniques to hundreds of breast cancer samples to provide a comprehensive catalog of somatic mutations that cause this disease (1–4). When these data are combined with a recent large-scale investigation of gene copy number aberration (CNA) linked to gene expression abnormalities (5), a comprehensive and highly complex picture of the somatic genetic events driving breast cancer pathogenesis is emerging. In the sixth article in this *Nature* 2012 Breast Cancer series, The Cancer Genome Atlas (TCGA) Network has taken the "omics" approach a step further by creating the largest sequence-based database (more than 500 exome sequences, with more in the pipeline) and by adding additional platforms to the now standard mutation/copy number/mRNA triad, including microRNA expression, DNA methylation to interrogate epigenetic regulation, and proteomic profiling using reverse phase protein arrays (RPPA; ref. 6). These data are now in the public domain and will be mined many times over as the complex process of biologic and clinical annotation proceeds. Table 1 provides a summary of the TCGA data with respect to the significantly mutated genes (SMG) in each of the 3 major clinical treatment categories of breast cancer. SMGs accumulate missense, nonsense, and small deletions or insertions at a rate that is above what would be expected by chance and, therefore, are likely to be mutational events that drive the disease process. Our discussion for this Prospective focuses on the implications of these data sets for systemic treatment of breast cancer (Fig. 1).

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TREATMENT CHALLENGES IN ESTROGEN RECEPTOR–POSITIVE/LUMINAL-TYPE BREAST CANCER

While in the node-negative setting, many patients with luminal-type disease do well if treated with endocrine drugs alone (tamoxifen or aromatase inhibitors); the risk of relapse extends up to 20 years or more after diagnosis. Risk of late relapse is a particularly significant problem for patients with node-positive luminal-type disease and for patients diagnosed at a younger age (7). In these settings, endocrine therapy alone is not an adequate standard. Furthermore, endocrine treatment involves multiple years of exposure to agents often difficult to tolerate that are prone to fail through the development of secondary resistance and/or lack of patient compliance. Unfortunately, standard adjuvant chemotherapy is also increasingly recognized as relatively, or even completely, ineffective against estrogen receptor–positive (ER^+)/luminal A–type breast cancer, as evidenced by retrospective analyses of the NSABP B-20 (8) and SWOG-8814 (9) trials, and by the low pathologic complete response rates seen with neoadjuvant chemotherapy (10). To evaluate this hypothesis, the treatment of patients with ER^+ disease and stratified by a 21-gene recurrence score-based gene expression profile (i.e., OncotypeDX) is being studied in 2 large clinical trials that randomly assign patients to chemotherapy and endocrine therapy or to endocrine therapy alone, (NCT01272037 and NCT00310180).

The development of safer and more effective targeted agents that could reduce late relapse would therefore be a considerable breakthrough. This goal will require more effective treatments to eradicate indolent disseminated tumor cells before endocrine resistance and progression mutations occur. ER^+/luminal B–type breast cancers have more complex genomes than do luminal A cancers, greater proliferative potential, and, despite often high levels of ER expression, less complete responses to endocrine therapy that translate into elevated relapse and death rates (4, 11, 12). Unfortunately, chemotherapy is still often ineffective in patients with high genomic-risk ER^+/luminal B cancer, and alongside basal-like breast cancer, luminal B disease is clearly one of the major unsolved problems in breast cancer oncology. Aside from the well-worked issue of the role of HER2 overexpression in driving some ER^+/luminal B tumors (13), explanations for the

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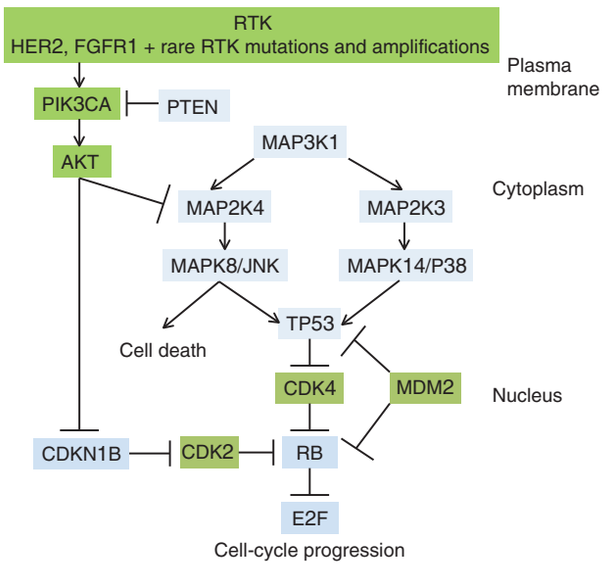

Figure 1. A simplified therapeutic roadmap based on somatic mutations in luminal-type breast cancer. This diagram is based on the analysis by Ellis and colleagues (4) of luminal-type breast cancers. All components outlined in the diagram were chosen based on evidence for either gene functional gain, indicated in green (e.g., amplification or gain-of-function mutation), or gene functional loss, indicated in blue (e.g. LOH, frameshift, or nonsense mutation). Matched therapeutic approaches for the gain-of-function events are discussed in Table 2. RTK, receptor tyrosine kinase.

failure to respond adequately to endocrine therapy despite ER expression have been inadequate.

The Phosphoinositide 3-Kinase Pathway in ER$^+$ Disease

On the basis of the mutational repertoire, PIK3CA mutation is the most common SMG in ER$^+$/luminal breast cancer , and its mutation is likely a causal genetic event, thereby making these events a top priority for therapeutic hypotheses (Table 1). The fact that *PIK3CA* is the most prevalent gain-of-function mutation after exhaustive unbiased sequencing of hundreds of breast cancers (~40% incidence), and that it is selectively the alpha catalytic subunit and not any of the other 3 Class I catalytic subunits, makes a powerful argument for highly specific PIK3CA targeting. Furthermore, even the site of the *PIK3CA* mutation may be subtype specific, as almost all of the "hotspot" E545K mutations observed in TCGA occurred in the luminal A subtype (25 of 27 cases; ref. 6). This finding suggests that adjuvant studies focused on the *PIK3CA*-mutant population may also need to stratify by mutation type, and by expression subtype, as relapse rates could be significantly different, depending upon these molecular features. Interestingly, the phosphoprotein signaling events produced by *PIK3CA* mutation seem subtle; the RPPA data in the TCGA analysis and earlier observations (14) do not show strong activation/phosphorylation of the canonical AKT/S6K proteins typically seen with PTEN loss, which is readily observed in basal-like breast cancers (6). Nevertheless, preclinical studies have shown that gene silencing or pharmacologic inhibition of PIK3CA induces marked apoptosis in *PIK3CA*-mutant ER$^+$ breast cancer, specifically under estradiol-deprived conditions or in the presence of an antiestrogen (15, 16). This finding argues for a synthetic lethal interaction between PIK3CA- and ER-targeting agents. The recent U.S. Food and Drug Administration approval of the rapamycin analogue everolimus [which inhibits mTOR, a downstream component of the phosphoinositide 3-kinase (PI3K) pathway], in combination with the steroidal estrogen–lowering agent exemestane for endocrine therapy of refractory advanced breast cancer, underscores the potential of targeting this pathway in ER$^+$ disease (17). Everolimus is, however, a fairly toxic drug that is poorly tolerated in some patients. Several PIK3CA-specific inhibitors are now in phase I or II clinical trials, with initial reports of efficacy in *PIK3CA*-mutant cases of luminal breast cancer (18). Other somatic mutations within the PI3K pathway also occur within ER$^+$/luminal cancers, but at a much lower frequency. These include mutation of *PTEN* (5%), activating mutations in *AKT1* (3%), and inactivating mutations in the regulatory subunit of the PI3K complex (*PIK3R1*–2%); thus, on the DNA sequence level alone, at least 4 possible DNA-based biomarkers of PI3K pathway activation exist and merit investigation in the context of drug studies (Table 1).

The Stress Kinase Pathway

Loss-of-function mutations in *MAP3K1*, an upstream activator of the stress-induced apoptotic kinase *c-jun*-NH_2-kinase (JNK), constituted one of the most selective genomic features of luminal disease (4, 6). These mutations often coexist with *PIK3CA* mutations and show a trend for mutual exclusivity, with mutations in *MAP2K4*, the immediate MAP3K1 downstream substrate in the JNK activation pathway (4, 6). Together, *MAP3K1* and *MAP2K4* mutations equaled approximately 12%, and it is also worth noting that *MAP2K4* mutation was frequently associated with loss of heterozygosity (LOH; refs. 5, 6) Through analysis of ER$^+$/luminal tumors undergoing neoadjuvant treatment with an aromatase inhibitor, *MAP3K1* mutations were shown to be associated with low grade, low proliferative index before and after estrogen deprivation therapy, and a luminal A gene expression pattern (4). Thus, *MAP3K1* mutation is a high-frequency driver mutation for luminal A disease and, therefore, also a likely causal molecular event. Of note, *PIK3CA* mutation activates AKT1, which in turn inhibits MAP2K4, thereby providing an additional pathway to attenuate JNK signaling even in wild-type *MAP2K4/MAP3K1* tumors. By adding together the common *PIK3CA* mutations, rarer PI3K pathway mutations (*AKT1*, *PTEN*, and *PIK3R1*), and *MAP3K1/MAP2K4*, one can reasonably state that the inhibition of JNK signaling is likely to be an initiating event in the majority of ER$^+$/HER2$^-$ cases (2, 6). The fact that these mutational events are strikingly lower in frequency, or even absent from basal-like breast cancers, also suggests that these events are fundamental to the difference between luminal and basal-like breast disease. Because an intact JNK pathway may be required for a cell death response to chemotherapy drugs (19), silenced or attenuated JNK signal transduction could explain one of the cardinal features of luminal A disease, namely, the relative insensitivity to standard chemotherapy regimens. Another genetic feature that may contribute to chemotherapy insensitivity is predominantly a wild-type TP53 pathway within ER$^+$/luminal A disease (9%–17% mutant; refs. 4, 6), which allows these cells to undergo cell-cycle arrest and DNA repair when challenged with DNA-damaging chemotherapeutics (20).

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Table 1. SMG lists for the 3 main breast cancer clinical categories from TCGA data

	ER$^+$/HER2$^-$ (n = 330)			Clinical HER2$^+$ (n = 75)			Triple negative (n = 86)		
Gene	Cases, n	LRT	CT	Cases, n	LRT	CT	Cases, n	LRT	CT
PIK3CA	145	0	0	23	0	0	9	5.55E-09	3.22E-10
TP53	68	0	0	41	0	0	68	0	0
GATA3	45	0	0	8	0	0	0	NA	NA
MAP3K1	36	0	0	2	NA	NA	0	NA	NA
CDH1	30	0	0	2	NA	NA	1	NA	NA
MLL3	28	0	0	5	NA	NA	3	NA	NA
MAP2K4	19	0	0	1	NA	NA	1	NA	NA
PTEN	16	0	0	0	NA	NA	1	NA	NA
RUNX1	15	0	0	1	NA	NA	0	NA	NA
NCOR1	13	1.10E-05	4.33E-07	1	NA	NA	1	NA	NA
TBX3	11	5.74E-12	4.91E-12	0	NA	NA	1	NA	NA
AKT1	11	2.75E-13	3.94E-12	1	NA	NA	0	NA	NA
CTCF	11	6.46E-04	2.31E-06	1	NA	NA	1	NA	NA
NF1	11	1.09E-02	1.39E-02	1	NA	NA	2	NA	NA
PIK3R1	9	8.44E-07	1.82E-06	4	NA	NA	1	NA	NA
FAM47C	8	8.08E-03	2.96E-02	1	NA	NA	0	NA	NA
CBFB	7	1.32E-07	5.10E-08	0	NA	NA	1	NA	NA
SF3B1	7	2.27E-03	1.07E-02	1	NA	NA	0	NA	NA
TBL1XR1	6	6.33E-04	1.83E-05	1	NA	NA	1	NA	NA
ZFP36L1	6	7.14E-05	1.27E-04	0	NA	NA	1	NA	NA
FOXA1	6	2.51E-02	8.19E-03	1	NA	NA	1	NA	NA
TLR4	6	4.54E-02	2.37E-02	1	NA	NA	0	NA	NA
CDKN1B	5	4.60E-06	5.94E-05	0	NA	NA	0	NA	NA
GPS2	4	6.98E-03	3.73E-02	1	NA	NA	1	NA	NA
OR6A2	4	3.67E-03	4.32E-03	0	NA	NA	0	NA	NA
OR2L2	4	1.01E-02	8.19E-03	0	NA	NA	0	NA	NA
RB1	3	NA	NA	1	NA	NA	4	2.77E-02	4.64E-02
PTPN22	3	NA	NA	4	6.57E-03	3.36E-02	0	NA	NA

NOTE: NA indicates that after statistical adjustment for background mutation rates and gene size, the given mutations observed were not considered statistically significant. The list is focused on missense, nonsense, and frameshift mutations, and small insertions and deletions (indels). Structural mutations (large deletions, inversions, translocations, and amplifications) were not taken into account. LRT refers to the likelihood ratio test and CT to the convolution test, both of which are measures of likelihood of whether observations could have occurred by chance.
Data in table from Koboldt et al. (6).

GATA3 and *RUNX1* Mutations

GATA3 mutation at approximately 10% frequency was noted in all 4 articles that studied a significant number of luminal tumors (2–4, 6). *GATA3* was the third most common mutation in TCGA overall, with 58 mutations, all but 3 of them occurring within ER$^+$/luminal tumors. Of note, all 13 hotspot CA intron 4 CA dinucleotide deletion mutants were in luminal A cases, whereas 7 of 9 exon 5 frameshift mutants were associated with luminal B cases (6). This finding suggests that the type of *GATA3* mutation may be an additional determinant of luminal A versus luminal B status, along with significant differences in copy number profiles (5, 6). Although it is not clear how one could target GATA3 directly, given that it is a transcription factor, studies of GATA3 in the neoadjuvant setting showed an association with greater responsiveness to aromatase inhibitors, suggesting an endocrine sensitivity phenotype (4). This observation suggests that study of the long-term outcome of

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ER$^+$ breast cancer according to *GATA3* status could produce clinically relevant data relating to questions such as the type of endocrine therapy that is most effective and the optimal duration of endocrine treatment. Loss-of-function mutations in *RUNX1*, and the gene encoding its dimerization partner CBFB, were both detected in ER$^+$/luminal disease (3, 4, 6). These genetic events are predicted to disrupt ER tethering to DNA at RUNX1/CBFB-binding sites (21) and may produce a phenotype opposite to that of *GATA3* mutation, namely, endocrine therapy resistance. In support of this hypothesis, mutations in *RUNX1* were associated in a PARADIGM-based pathway informatics model (22) with luminal B disease (4).

MLL3 Mutations and a Putative Link to Histone Deacetylase Inhibition

Another feature of the SMG list in ER$^+$/HER2$^-$ breast cancer is the presence of additional genes previously implicated in leukemia and myelodysplasia. Besides *RUNX1* and *CBFB*, both of which are linked to the M2 subtype of acute myelogenous leukemia, mutations in the mixed lineage leukemia gene *MLL3* (as well as lower frequency mutations in other family members such as *MLL2*) were observed in several of the sequencing studies (2, 4, 6). MLL family members encode histone trimethyltransferases, considered positive global regulators of gene transcription, with functions that include regulation of ER gene expression (23). Theoretically, *MLL*-mutant tumors, as well as others with mutations in genes that are epigenetic regulators of gene expression, might be sensitive to the burgeoning class of drugs that target posttranslational histone modifications (24). Connectivity mapping showed that histone deacetylase (HDAC) inhibitors should be active in t(4; 11)-positive (MLL3 fusion gene driven) infant acute lymphoblastic leukemia (25); it is therefore of interest that randomized phase II data for the HDAC inhibitor entinostat showed activity in ER$^+$ advanced breast cancer in combination with the aromatase inhibitor exemestane (26). It is also worth mentioning in the context of epigenetic events that there was a "subtype" of luminal B tumors that was characterized by hypermethylated DNA, and that showed a significant reduction in *PIK3CA* mutation frequency; thus, it is possible that aberrant DNA methylation, and presumed inactivation of some yet to be determined gene(s), may mimic PIK3CA activation, which seems to be a requisite event for ER$^+$/luminal disease. This idea raises the possibility of treating a subset of luminal B tumors with drugs that target DNA methylation abnormalities.

The Role of p53 and p53 Regulators (MDM2) in Determining Luminal B Status and Endocrine Therapy Resistance

Luminal-type breast cancer is an unusual epithelial malignancy, as the majority of tumors, even when luminal B is considered, do not harbor mutant *TP53*. However, when mutant *TP53* is present, it is strongly associated with luminal B status and endocrine therapy resistance (4). Bioinformatics analysis using PARADIGM to integrate gene CNA, gene expression, and mutation status (22) clearly identifies a p53 endocrine therapy resistance hub (4). In the TCGA analysis, approximately 30% of luminal B tumors were *TP53* mutant, and interestingly, an equivalent number exhibited amplification of the p53 antagonist MDM2 (6), thus suggesting that the majority of luminal B tumors are p53 pathway defective. The interaction between MDM2 and p53 regulates baseline protein levels and activity of p53 through an autoregulatory feedback loop (27, 28). MDM2 directly inhibits the transactivation function of p53, exports p53 out of the nucleus, and promotes proteosome-mediated degradation of p53 through its E3 ubiquitin ligase activity (27). Thus, inactivation of p53 by mutation, by amplification of MDM2 or MDM4, or by rarer mutations in other components of the p53 pathway (ATM, CHEK2) are likely to be causal events of the luminal B phenotype. In the past few years, orally bioavailable small-molecule inhibitors of the MDM2–p53 interaction have been developed (27, 29–31). These agents dramatically increase wild-type p53 levels and thereby trigger cell-cycle arrest and apoptosis in cancer cells (29–31). At least one potent analogue (RO5503781) is now in phase I clinical development (NCT01462175), and thus, MDM2 inhibition is a promising approach for the treatment of wild-type TP53 luminal B disease.

CDK4/6 Inhibitors for Luminal B Tumors?

The TCGA breast cancer study also showed low levels of *RB1* mutations in luminal-type breast cancer (~1%) and frequent amplification of *cyclin D1* (40%; ref. 6). The cyclin D1/CDK4/6 complex phosphorylates the retinoblastoma (Rb) protein, which leads to cell-cycle activation (32). Results from several studies indicate that *CDK4* and *CDK6* play an important role in estrogen-stimulated proliferation of breast cancer cells in early to mid G_1 phase (33–37). Thus, CDK4 and CDK6 represent valuable therapeutic targets of ER$^+$ advanced breast cancer. Consistent with this conclusion, high levels of expression of the Rb-regulated *E2F* gene family is a frequent feature of endocrine-resistant luminal-type breast cancer (38). Unlike basal-like breast cancers, in which Rb loss is common (39), inhibition of the critical cell-cycle inhibitory effect of Rb in luminal tumors is achieved primarily through overexpression or amplification of cyclin D1, overexpression or amplification of CDK4, and/or loss of the CDK inhibitors CDKN1B, CDKN2A, and 2B (4, 6). Luminal tumors are therefore likely to prove sensitive to CDK4/6 inhibitors as randomized phase II trials are under way (NCT00721409), and a preliminary report of a randomized trial of letrozole versus letrozole plus PD0332991 at the 2012 IMPAKT meeting in Brussels showed significant improvement in progression-free survival (40). Interestingly, mantle zone lymphoma, another chemotherapy-refractory cyclin D–driven malignancy, is also sensitive to CDK4/6 inhibition (41). Basal-like tumors, and occasional luminal B tumors, harbor loss or mutation in Rb and are therefore highly unlikely to be unresponsive to these agents; thus, determination of the mode of Rb pathway inactivation will be critical to the success of CDK 4/6 inhibitors in breast cancer (42).

BASAL-LIKE BREAST CANCER—LINKS TO HIGH-GRADE SEROUS OVARIAN CANCER

Luminal-type and basal-like breast cancers were clearly completely different diseases on all levels of data analysis (1, 5, 6). Basal-like breast cancers show more similarity with other tumors arising in the basal layer of the epidermis, such as squamous carcinoma of the lung or head and neck, as well

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as the epithelium of the ovary. Of note, these are platinum-sensitive diseases, yet platinum-based chemotherapy is not a standard of care for basal-like breast cancer because current breast cancer chemotherapy regimens were developed in unselected populations of patients with breast cancer, 70% to 80% of which are luminal disease. This situation has led to an overemphasis on anthracycline-based treatment, which may be effective only in clinically HER2$^+$ disease, and perhaps only in tumors with the HER2-enriched (HER2-E) gene expression phenotype (i.e., clinically HER2$^+$ and HER2-E; ref. 43). A trial to compare standard anthracycline/cyclophosphamide/taxane-based chemotherapy with a platinum/taxane-based approach specifically in basal-like breast cancer is unquestionably a high-priority clinical trial suggested by the TCGA results (6). From a somatic mutation perspective, *TP53* is the only gene recurrently mutated in basal-like disease at a frequency of more than 10%, and it showed an 85% mutation frequency in the TCGA data. This tremendously high mutation frequency, along with the lack of other frequently mutated genes, mirrors the results seen in serous ovarian cancers (44), and again shows commonalities between these 2 anatomically distinct tumor types. The presence of *BRCA1* and *BRCA2* mutations, both germline and somatic, in basal-like breast cancer (which totaled ~20% frequency when combined) is another link to ovarian cancer and a clear therapeutic opportunity with respect to the use of PARP inhibitors (45); clinical investigations of the PARP inhibitor velaparib, in combination with carboplatin and paclitaxel, are already under way (NCT01506609). In addition, although few somatic mutation targets exist in basal-like (and ovarian cancers) besides *BRCA1*/2, a plethora of amplification and deletion candidates (see below) does exist.

The PI3K Pathway in Basal-like Breast Cancer

A strong genomic and proteomic signature of active PI3K pathway activity is found within basal-like breast cancers, and this represents a major druggable pathway. However, the pathway activation events are different relative to ER$^+$/luminal disease, which may require a distinct pharmacologic approach. Unlike ER$^+$/luminal-type breast cancer, in which *PIK3CA* mutation dominates, PI3K pathway–activating events in basal-like tumors include a much broader spectrum of genes, with a lower frequency of *PIK3CA* mutation (9%) and more frequent deletion, mutation, or loss of negative regulators such as *PTEN* (35%) and *INPP4B* (30%; ref. 6). Furthermore, the *MAGI3–AKT3* gene fusion is a unique addition to the list of basal-like/triple-negative breast cancer (TNBC)–activating mutations (3). The PI3K pathway RPPA data in TCGA (6), as well as multiple transcriptional signatures, show a strong activation signature in the majority of basal-like cases, which could be used as a summary readout of the spectrum of upstream-activating genetic events. Thus, numerous biomarkers of PI3K pathway activation exist, and many could prove clinically useful if validated by clinical investigation (6).

HER2$^+$ BREAST CANCER—MORE THAN ONE SUBTYPE

Clinically, HER2$^+$ disease is also a heterogeneous group, with approximately 50% of cases responding to HER2-targeted therapies. From the TCGA data, clinical HER2$^+$ disease was clearly at least 2 groups, with strong links between DNA, RNA, and protein-based analysis. One HER2$^+$ subgroup was associated with high levels of EGF receptor (EGFR) and HER2 protein phosphorylation and a tendency to be ER$^-$, whereas the second group had lower level DNA amplification and lower protein-based signaling, and tended to be ER$^+$/luminal. The clinical phenotypes associated with this distinction now need to be evaluated on clinical trials of HER2 inhibitors like trastuzumab and/or lapatinib, as the TCGA data suggest that these cell lineage or proteomic distinctions may be biomarkers of trastuzumab and/or lapatinib sensitivity. Indeed, late-breaking data at the European Society for Medical Oncology (ESMO) Vienna 2012 congress on the duration of adjuvant trastuzumab therapy hint at a difference between HER2$^+$/ER$^+$ and HER2$^+$/ER$^-$ disease, in keeping with the concept that HER2$^+$/luminal is biologically distinct from HER2$^+$/HER2-enriched disease, which is predominantly ER$^-$ (10). From a somatic mutation perspective, clinical HER2$^+$ disease was similar to basal-like disease in that the majority of cases were TP53-mutant (~75%). In addition, a few other somatic mutation alterations frequently occurred, including PIK3CA (30%) and another PI3K pathway component (*PIK3R1*–5%). Interestingly, in the TCGA data set, eight-HER2 somatic-mutant tumors were identified, of which 4 were described as the lobular histologic subtype; only 1 of 8 of these cases also had *HER2* amplification. Low-frequency *HER2* mutations were also noted in 2 of the other articles (1, 4). Thus, the identification of these *HER2*-mutant (but not amplified) cases may be a subgroup that might benefit from *HER2*-targeted therapies, and functional/pharmacologic data are forthcoming from TCGA investigators. While *HER2* mutations occur in only 1% to 2% of breast cancers, breast cancer is so common that a 2% population frequency produces a study population comparable with that of chronic myeloid leukemia (about 4,000 cases/year), a disease for which oral tyrosine kinase inhibitors are standard and randomized trials have been conducted. Thus, although energetic mutation screening programs will be required for low-frequency druggable mutations, these leads should be aggressively pursued if the preclinical biology and pharmacology are compelling.

TARGETING OTHER RECEPTOR TYROSINE KINASES ACTIVATED BY GENE AMPLIFICATION

HER2 amplification is an established biomarker of drug sensitivity, and thus the amplification of other RTKs may also provide drug sensitivity biomarkers. Within basal-like, luminal B, and HER2$^+$ disease, all of which were genomically unstable, many DNA copy number amplifications were present (5, 6). Many of the most commonly amplified genes are associated with existing drugs, including those encoding EGFR, c-KIT, PDGFRA and B, FGFR1–4, FGF ligands, and c-MET. A key question concerning these genes and proteins is whether the amplification (without mutation) predicts drug sensitivity. A convincing case is developing for *FGFR1* amplification, which is a known luminal B driver (46). A high response rate to the fibroblast growth factor receptor (FGFR) and VEGF receptor (VEGFR) inhibitor E-3810,

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Table 2. Examples of mutation-matched therapies for breast cancer

Altered genes with predictive biomarker potential	Treatment approach	Strength of hypothesis for somatic alteration–targeted drug match (reference)
HER2 amplification	HER2-directed antibodies and HER2 kinase inhibitors	1 Trastuzumab, pertuzumab, and lapatinib. All approved agents
PIK3CA mutation	PIK3CA-selective inhibitors	2 Phase I BYL719 (18)
FGFR1 amplification, *FGF3* amplification, other FGF ligands and receptors, and rare receptor mutations	FGFR small-molecule inhibitors and antibodies	2 Phase I BGJ398 (48) and phase I E3800 (47)
Inherited and somatic *BRCA1* and *BRCA2* mutation	PARP inhibitors	2 Olaparib (49) and veliparib: NCT01506609[a]
Cyclin D1/CDK4/CDK6 amplification or deletion of *CDKN1B*, CDKN2A, and CDKN2B	CDK4/6 inhibitors	2 PD0332991 (40)
AKT1–3 gain-of-function mutation/gene fusion via translocation/amplification	AKT inhibitors	3 MK-2206: NCT01277757[a]
GATA3 mutation	Aromatase inhibition	3 Retrospective analysis of Z1031 (4)
PTEN/INPP4B loss-of-function mutation/deletion/loss of expression in TNBC	Broad-spectrum PI3K pathway inhibitors	3 BKM120: NCT01629615[a]
MDM2 amplification in *TP53* wild-type tumors	MDM2 inhibitors	3 RO5503781: NCT01462175[a]
HER2 mutation	Small-molecule *HER2* kinase inhibitors	3 Neratinib: (NCT01670877)[a] (50)
PIK3R1 loss-of-function mutation	PI3K pathway inhibitors?	4
MLL family member mutation	HDAC inhibition?	4
Rare RTK mutations	Various matched inhibitors?	4

NOTE: Number 1 indicates approved therapy; 2, early evidence of efficacy; 3, clinical investigations under way; and 4, clinical investigations not yet activated.
[a]Clinical Trial.gov number. The trials mentioned in this table are examples, and the list is not meant to be comprehensive.

specifically in *FGFR1*-amplified breast cancer, was recently reported at ESMO 2012 (47), supporting reports of this drug class in other settings (48). The current state of the FGFR field underscores the importance of highly active agents and upfront selection of patients for clinical success when investigating druggable amplified targets identified though genomic screens.

TARGETING RARE KINASE MUTATIONS

Somatic mutations in kinases other than PIK3CA and AKT1 were rare, but a few have been observed. For example, a number of *BRAF* mutations were identified in the TCGA study. However, none of them were the well-known V600E variant that is the target of successful therapy for patients with melanoma, although a K601E mutation was observed in the Washington University Genome Institute Luminal Breast Cancer Project (4). This finding illustrates a major challenge for personalized medicine, namely, what does one do with rare, potentially druggable mutations that occur in fewer than 1% of cases, and, for which, the functional consequences of the mutation may not be obvious or previously studied? Perhaps computational predictions, or even "one off" *in vitro* functional characterizations, will be required before a decision to give a mutation-matched drug can be made. Additional kinases with previously known pathogenic mutations in other cancer types were detected in breast cancers, including somatic variants in Janus-activated kinase (JAK) 1, JAK2, and anaplastic lymphoma kinase (ALK), but again, the mutations found in these patients with breast cancer were not the type seen within the other cancer disease types. Activating mutations in *HER2* have recently emerged as a more promising possibility. In a database of 1,500 cases, *HER2* mutations have been found in 1.6% of the cases. Because breast cancer is so common, this produces a study population of over 4,000 cases a year, comparable with that for chronic myeloid leukemia, for example. A phase II trial of neratinib in *HER2* mutant has been activated (50).

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CONCLUSIONS

The combined knowledge base provided by these 6 recent breast cancer genomics papers is unprecedented, and it will take many years before all the therapeutic hypotheses raised by this vast data repository will be addressed. Nonetheless, new therapeutic roadmaps are emerging, and the opportunities in luminal-type breast cancer are particularly compelling (Fig. 1 and Table 2). It therefore seems increasingly likely that a new treatment paradigm is rapidly evolving whereby deep genomic analysis will drive treatment decisions based on a pharmacopeia of cell-type and pathway-matched therapies. Perhaps the term "personalized therapy" is overused. After all, does a hematologist, when working up a case of anemia, use the term "personalizing therapy" for the treatment of low hemoglobin level? Rather, the workup is focused on making the correct diagnosis from a long list of more than 100 acquired and inherited causes. Similar to "anemia," the term "breast cancer" is a symptom complex, an abnormal proliferation of invasive cells in the breast, not a true etiology-based diagnosis. However, a "real" diagnosis and appropriate etiology-matched treatment, based on identification of driving genetic events within the context of the tumor cell type of origin (i.e., luminal vs. basal), is now an immediate prospect.

Disclosure of Potential Conflicts of Interest

M.J Ellis serves as a board member and has an ownership interest (including patents) in University Genomics and Bioclassifier LLC. C.M Perou serves as a board member and has an ownership interest (including patents) in University Genomics and Bioclassifier LLC.

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CANCER DISCOVERY

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