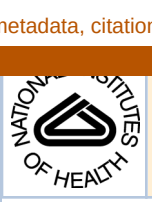

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The Genomic Landscape of Breast Cancer as a Therapeutic Roadmap

Matthew J. Ellis and
Division of Medical Oncology, Section of Breast Oncology, Washington University School of Medicine, Siteman Cancer Center, 660 South Euclid Ave, CB 8069, St Louis, MO, 63110, Ph: 314-362-8903, Fax: 314-747-9320

Charles M. Perou
Department of Genetics, University of North Carolina, Lineberger Comprehensive Cancer Center, 450 West Drive, CB 7295, Chapel Hill, NC, 27599, Ph: 919-843-5740, Fax: 919-843-5718

Matthew J. Ellis: mellis@dom.wustl.edu; Charles M. Perou: cperou@med.unc.edu

Abstract

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 etiological events that drive breast cancer are finally coming into focus and should be used to set priorities for clinical trials. In this Research Focus we summarize some of the headline conclusions from six recent breast cancer 'omics profiling' papers in Nature, with an emphasis on the implications for systemic therapy.

Introduction

Over the last six months, four papers have appeared in Nature that describe the application of massively parallel sequencing techniques to hundreds of breast cancer samples in order 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 paper 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 data base (over 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)[6]. These data are now in the public domain and will be mined many times over as the complex process of biological 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 three major clinical treatment categories of breast cancer. SMG 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 commentary focuses on the implications of these data sets for systemic treatment of breast cancer.

Correspondence to: Matthew J. Ellis, mellis@dom.wustl.edu.

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Treatment challenges in estrogen receptor positive (ER+)/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 often difficult to tolerate agents that are prone to fail through the development of secondary resistance and/or lack of patient compliance. Unfortunately standard adjuvant chemotherapy is also increasing recognized as relatively, or even completely, ineffective against ER+/Luminal A-type breast cancer as evidenced by retrospective analyses of NSABP B-20[8] and SWOG-8814[9] trials, and by the low pathological 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 two large clinical trials that randomize patient treatment to chemotherapy and endocrine therapy or 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 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 Luminal A, 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 these high genomic risk ER+/Luminal B patients, 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 over-expression driving some ER+/Luminal B tumors[13], explanations for the failure to respond adequately to endocrine therapy despite ER expression have been inadequate.

The phosphatidylinositol-3-kinase (PI3K) pathway in ER-positive disease—

Based on the mutational repertoire, PIK3CA mutations are the most common SMG in ER+/Luminal breast cancer and 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 three 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 (25/27)[6]. This suggests that adjuvant studies focused on a 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 phospho-protein signaling events produced by PIK3CA mutation appear subtle; the RPPA data in the TCGA analysis and earlier observations[14] does 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]. Despite this, preclinical studies have demonstrated that gene silencing, or pharmacological 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 argues for a synthetic lethal interaction between PIK3CA and ER targeting agents. The recent FDA approval of the rapamycin analog everolimus (which inhibits mTOR - a

downstream component of the PI3-kinase pathway), in combination with the steroidal estrogen-lowering agent exemestane for endocrine therapy 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 1 or 2 clinical trials with initial reports of efficacy in PIK3CA mutant cases of luminal breast cancer[18]. Other somatic mutations within the PI3 kinase pathway also occur within ER+/Luminal cancers, but at a much lower frequency. These included 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 (JNK) Pathway—Loss of function mutations in MAP3K1, an upstream activator of the stress-induced apoptotic kinase JUN terminal kinase (JNK), was one of the most selective genomic features of Luminal disease[4, 6]. These mutations often coexist with PI3KCA mutations and show a trend for mutual exclusivity with mutations in MAP2K4, the immediate MAP3K1 down-stream substrate in the JNK activation pathway [4, 6]. Together, MAP3K1 and MAP2K4 mutations equaled ~12%, and it is also worth noting that MAP2K4 was frequently associated with LOH[5, 6] By analyzing 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 MAP3K1mutation 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 *wt* 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 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. Since 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 *wt* TP53 pathway within ER+/Luminal A disease (9–17% mutant)[4, 6], which allows these cells to undergo cell cycle arrest and DNA repair when challenged with DNA damaging chemotherapeutics[20].

GATA3 and RUNX1 mutations—GATA3 mutation at ~10% frequency was noted in all four papers that studied a significant number of luminal tumors[2–4, 6]. GATA3 was the third most common mutation in TCGA overall with 58 mutations, with all but 3 occurring within ER+/Luminal tumors. Of note, all 13 hotspot CA intron 4 deletion mutants were in Luminal A cases, while 7/9 exon 5 frame-shift mutants were associated with Luminal B cases[6]. This 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]. While it is not clear how one could target GATA3 directly given that it is a transcription factor, studies of GATA3 in the neoadjuvant setting demonstrated 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 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 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 GATA3 mutation, namely endocrine therapy resistance. In support of this hypothesis, mutations in RUNX1 were associated in a PARADIGM[22]-based pathway informatics model with Luminal B disease[4].

MLL3 mutations and a link to HDAC 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 AML, mutations in the mixed lineage leukemia gene MLL3 (as well as lower frequency mutations in other family members such as MLL2) was 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 estrogen receptor 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 post-translational histone modifications[24]. Connectivity mapping demonstrated that 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 2 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 hyper-methylated DNA, and which 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 raises the possibility of treating a subset of Luminal B tumors with drugs that target DNA methylation abnormalities.

The role of TP53 and TP53 regulators (MDM2) in determining Luminal B status and endocrine therapy resistance—Luminal-type breast cancer is an unusual common 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 TP53 endocrine therapy resistance hub[4]. In the TCGA analysis, ~30% of Luminal B tumors were TP53 mutant, and interestingly an equivalent number exhibited amplification of the TP53 antagonist MDM2[6], thus suggesting the majority of Luminal B tumors are TP53-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 TP53 either by mutation, amplification of MDM2 or MDM4, or rarer mutations in other components of the TP53 pathway (ATM, CHEK2) are likely to be causal events of the Luminal B phenotype. In the last 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 therefore a promising approach for the treatment of *wt* TP53 Luminal B disease.

CDK4/6 inhibitors for Luminal B tumors?—The TCGA breast cancer study also demonstrated low levels of RB1 mutations in Luminal-type breast cancer (~1%) and frequent amplification of Cyclin D1(40%)[6]. The Cyclin D1/CDK4/6 complex phosphorylates the retinoblastoma (Rb) protein, which leads to cell cycle activation[32]. Studies indicate that CDK4 and CDK6 play an important role in estrogen-stimulated proliferation of breast cancer cells in early to mid-G1 phase [33–37]. Thus, CDK4 and CDK6 represent valuable therapeutic targets of ER-positive 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 where Rb loss is common[39], inhibition of the critical cell cycle inhibitory effect of Rb in Luminal tumors is achieved primarily through over expression/amplification of Cyclin D1, over-expression/amplification of CDK4, and/or loss of the CDK inhibitors CDKN1B, CDKN2A and 2B[4, 6]. Luminal tumors are therefore likely to be sensitive to CDK4/6 inhibitors and randomized Phase 2 trials are underway (NCT00721409) and a preliminary report of a randomized trial of letrozole versus letrozole plus PD PD0332991 at the 2012 IMPAKT meeting in Brussels showed significant improvement in progression-free survival[40]. Interestingly mantel 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/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 cancer were clearly completely different diseases on all levels of data analysis[1, 5, 6]. Basal-like breast cancer show more similarity with other tumors arising in the basal layer of the epidermis, such as squamous carcinoma of the lung, head and neck, as well 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 breast cancer patients, 70–80% of which are Luminal disease. This has led to an over emphasis on anthracycline-based treatment, which may only be effective 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)[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 greater than 10%, and showed an 85% mutation frequency in the TCGA data. This tremendously high mutation frequency, and the lack of other frequently mutated genes, mirrors the results seen in Serous Ovarian cancers[44], and again demonstrates commonalities between these two anatomically distinct tumor types. The presence of BRCA1 and 2 mutations, both germ-line 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 utilization of PARP inhibitors[45] with clinical investigations of the PARP inhibitor Velaparib, in combination with carboplatin and paclitaxel already underway (NCT01506609). Also, while there are few somatic mutation targets in Basal-like (and ovarian cancers) besides BRCA1/2, there is a plethora of amplification and deletion candidates (see below).

The PI3 kinase pathway in Basal-like Breast Cancer—There is a strong genomic and proteomic signature of active PI3K-pathway activity 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 pharmacological approach. Unlike ER+/Luminal-type breast cancer where 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/loss of negative regulators such as PTEN (35%) and INPP4B (30%)[6]. Furthermore the MAGI3-AKT3 gene fusion is a unique addition to the list of Basal-like/TNBC activating mutations[3]. The PI3K-pathway RPPA data in TCGA[6], as well as multiple transcriptional signatures, shows 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 positive breast cancer – more than one subtype

Clinically HER2-positive disease is also a heterogeneous group, with approximately 50% 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. One HER2+ subgroup was associated with high levels of EGFR and HER2 protein phosphorylation and a tendency to be ER-negative, while the second group had lower level DNA amplification, lower protein-based signaling and tended to be ER+/Luminal. The clinical phenotypes associated with this distinction now needs to be evaluated on clinical trials of HER2 inhibitors like trastuzumab and/or lapatinib, as the TCGA data suggest that these cell lineage/proteomic distinctions may be biomarkers of trastuzumab and/or lapatinib sensitivity. Indeed late breaking data at the ESMO Vienna 2012 congress on the duration of adjuvant trastuzumab therapy hints 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, there were few other frequent somatic mutation alterations, including PIK3CA (30%) and another PI3K-pathway component (PIK3R1-5%). Interestingly, in the TCGA data set, 8 HER2 somatic mutant tumors were identified, of which 4 were described as the lobular histological subtype; only one out of eight of these cases also had HER2 amplification. Low frequency HER2 mutations were also noted in two of the other papers [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/pharmacological data are forthcoming from TCGA investigators. While HER2 mutations only occur in 1–2% of breast cancer, breast cancer is so common that a 2% population frequency produces a study population comparable to chronic myeloid leukemia (~4000 cases/year), a disease for which oral tyrosine kinase inhibitors are standard and randomized trials have been conducted. Thus while 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 Receptor Tyrosine Kinases (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 EGFR, c-

KIT, PDGFRA and B, FGFR1-4, FGF ligands, and c-MET. A key question concerning these genes/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 FGFR and VEGFR inhibitor E-3810 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 patient selection 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 were the well known V600E variant that is the target of successful therapy for melanoma patients, 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 less 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 known previously known pathogenic mutations in other cancer types were detected in breast cancers including somatic variants in JAK1, JAK2, and ALK, but again, the mutations found in these breast cancer patients were not the type seen within the other cancer disease types.

Summary

The combined knowledge base provided by these six 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 road maps are emerging and the opportunities in luminal-type breast cancer are particularly compelling (Figure 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 work up is focused on making the correct diagnosis from a long list of over 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 versus basal), is now an immediate prospect.

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Biographies

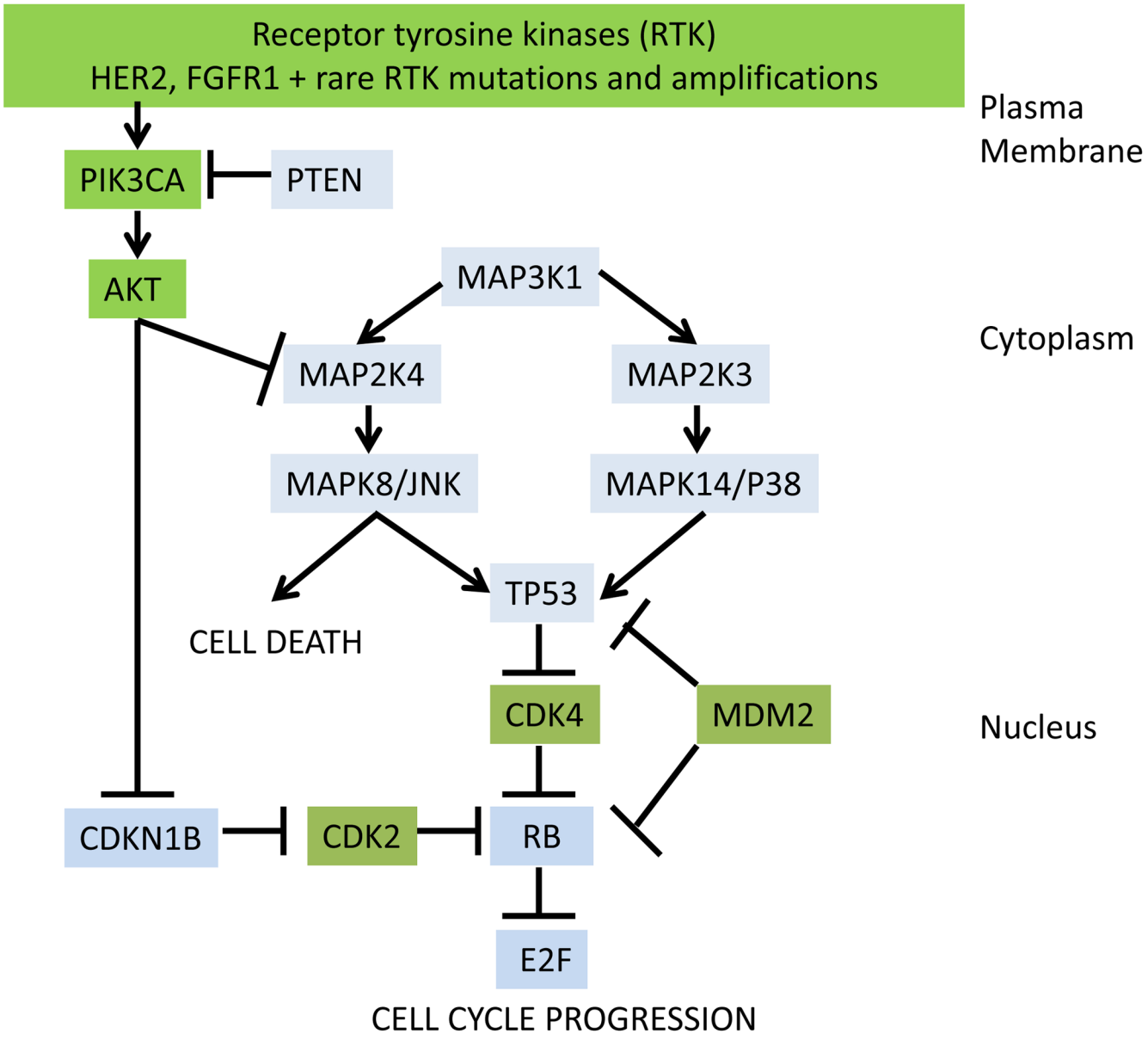

Figure 1.

A simplified therapeutic road map based on somatic mutations in luminal-type breast cancer. This cartoon is based on the analysis by Ellis et al of luminal type breast cancers[4]. All components outlined in the cartoon 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.

Table 1

The Significantly Mutated Gene (SMG) lists for the three main breast cancer clinical categories from the TCGA data[6].

Gene	ER+/HER2-(n=330)			Clinical HER2+ (n=75)			Triple-negative (n=86)		
	#Cases	LRT	CT	#Cases	LRT	CT	#Cases	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

Gene	ER+/HER2-(n=330)			Clinical HER2+ (n=75)			Triple-negative (n=86)		
	#Cases	LRT	CT	#Cases	LRT	CT	#Cases	LRT	CT
PTPN22	3	NA	NA	4	6.57E-03	3.36E-02	0	NA	NA

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 frame-shift mutations, and small insertions and deletions (indels). Structural mutations (large deletions, inversions, translocations and amplifications) were not taken into account.

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*
HER2 amplification	HER2 directed antibodies and HER2 kinase inhibitors	1 Trastuzumab, Pertuzumab and Lapatinib. All approved agents
PIK3CA mutation	PIK3CA selective inhibitors	2 Phase 1 BYL719[18]
FGFR1 amplification, FGF3 amplification, other FGF ligand and receptors, rare receptor mutations	FGFR small molecule inhibitors and antibodies	2 Phase 1 BGJ398[48] Phase 1 E3800[47]
Inherited and somatic BRCA1 and BRCA2 mutation	PARP inhibitors	2 Olaparib[49] Veliparib: NCT01506609†
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†
GATA3 mutation	Aromatase inhibition	3 Retrospective analysis of Z1031[4]
PTEN/INPP4B – loss of function mutation/ deletion/loss of expression in TNBC	Broad Spectrum PIK3 pathway inhibitors	3 BKM120: NCT01629615†
MDM2 amplification in TP53 wt tumors	MDM2 inhibitors	3 RO5503781: NCT01462175†
PIK3R1 – loss of function mutation	PIK3 pathway inhibitors?	4
MLL family member mutation	HDAC inhibition?	4
Rare RTK mutations	Various matched inhibitors?	4

1: Approved Therapy, 2: Early evidence of efficacy, 3: Clinical investigations underway, 4: Clinical investigations not yet activated.

†Clinical Trial.gov number.

The trials mentioned in this table are examples, and the list is not meant to be comprehensive.