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Review

Developmental therapeutics for inflammatory breast cancer: Biology and translational directions

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ABSTRACT

Inflammatory breast cancer (IBC) is a rare and aggressive form of breast cancer, which accounts for approximately 3% of cases of breast malignancies. Diagnosis relies largely on its clinical presentation, and despite a characteristic phenotype, underlying molecular mechanisms are poorly understood. Unique clinical presentation indicates that IBC is a distinct clinical and biological entity when compared to non-IBC. Biological understanding of non-IBC has been extrapolated into IBC and targeted therapies for HER2 positive (HER2+) and hormonal receptor positive non-IBC led to improved patient outcomes in the recent years. This manuscript reviews recent discoveries related to the underlying biology of IBC, clinical progress to date and suggests rational approaches for investigational therapies.

INTRODUCTION

Inflammatory breast cancer (IBC) is a rare and aggressive phenotype of breast cancer encompassing approximately 3% of newly diagnosed breast tumors [1]. IBC tends to affect younger women when compared to locally advanced non-IBC with a median age at diagnosis of 57 years [2]. Among African Americans the annual incidence is estimated at 3 (2.8-3.2) per 100,000 compared to 2.1(2.1-2.2) per 100,000 among white women [3]. IBC has no histological diagnostic criteria and its diagnosis is based primarily on its clinical presentation. In 1956, Haagensen et al. described diagnostic criteria for IBC including a rapidly enlarging breast, erythema involving at least one-third of the breast, generalized induration, and biopsy proven carcinoma [4]. These remain the cornerstone diagnostic criteria for IBC [5]. IBC is also characterized by distinct skin changes including diffuse erythema and edema (*peau-d'orange*) often without a clinically evident underlying mass, which is presumed to be secondary to lymphangiogenesis and tumor emboli in the lymphatics [6–8]. It usually has an abrupt onset and rapid progression, with a high risk of axillary lymph node involvement and distant metastases at the time of initial diagnosis [9]. Despite multimodality therapy, survival rates are lower than those for non-IBC. Patients with IBC have poorer prognosis when compared to locally advanced non-IBC (5-year overall survival rates of 62% versus 81%) [10–12]. Its distinct clinical presentation and aggressive course indicate that IBC is in fact a distinct biological entity. Furthermore, the majority of newly diagnosed cases are at least stage III with approximately 30% of *de novo* stage IV [11]. Moreover, IBC has been associated with high incidence of micrometastatic disease, defined by detection of either bone marrow metastasis or circulating tumor cells (CTCs) suggesting difficulty in clinical outcome comparison with non-IBC and potential need for extended treatment beyond standard multimodality treatment [13, 14].

The standard management of IBC involves a multimodality approach, including primary systemic chemotherapy followed by mastectomy, axillary lymph node dissection, and radiation therapy, which has led to improved survival outcomes [15, 16]. The National Comprehensive Cancer Center (NCCN) guidelines list the standard approach to IBC as neoadjuvant chemotherapy with an anthracycline based regimen and a taxane [17].

HER2 is a transmembrane receptor which when overexpressed stimulates a multitude of growth factor signaling pathways in breast cancer cells [18]. IBC is associated with higher prevalence of over-expression of HER2 when compared to non-IBC with estimates ranging from 40-58% [11, 19–23]. If HER2 is overexpressed, chemotherapy with trastuzumab in combination with pertuzumab is indicated as part of the systemic preoperative regimen. Mastectomy with axillary lymph node dissection is standard in IBC patients who respond to pre-operative chemotherapy. Following surgery, postmastectomy radiation including the chest wall and supraclavicular nodes plus or minus the internal mammary nodes is recommended, as well as adjuvant endocrine therapy if indicated.

Further understanding of the molecular biology of non-IBC has led to significant advances in the treatment of breast cancer, which in conjunction with improved screening strategies, Have increased survival rates [24–26]. For instance, the comprehensive genomic analysis of breast cancer through The Cancer Genome Atlas (TCGA) program supports not only that breast cancer is a heterogeneous disease but drug development strategies should take in to account molecular aberrations specific of each subtype [27]. An increased understanding of IBC biology has been relatively hampered by its rarity and maybe more importantly by its underdiagnosis or misdiagnosis as a consequence of subjective diagnostic criteria [28]. Researchers are attempting to better characterize the molecular biology of IBC, in hopes that this will ultimately guide developmental therapeutics efforts for this rare form of breast caner. This information combined with the current understanding about the aggressive clinical presentation, high frequency of micrometastatic disease and early recurrence rates can provide leads to improved therapies. In addition, clinical trial design should also take into account the positive correlation between pathologic complete response rate (pCR) with disease-free survival benefit for a subset of patients with IBC (i.e.: HER2+ IBC) as trials assessing the efficacy of neoadjuvant treatments are inherently shorter [29]. This manuscript aims to review the discoveries in the biology of IBC while highlighting the rationale for developmental therapeutics approaches.

GENOMIC ABERRATIONS IN IBC VS. NON-IBC

Besides differences observed from oncoprotein and gene copy number analyzes (e.g. HER2 expression/amplification) RNA next generation sequencing (NGS) technologies have allowed for better understanding of the mutational landscape of IBC [30]. A seminal work was conducted under the auspices of the IBC World Consortium by Van Laere et al. who reported results of Affymetrix gene expression profiling and molecular classification using the PAM50-algorithm derived from 137 patients with IBC and 252 patients in non-IBC [31]. Four robust IBC-sample clusters were identified, associated with the different molecular subtypes ($p < 0.001$), all of which were identified in IBC with a similar prevalence as in non-IBC, except for the luminal A subtype (19% in IBC vs. 42% in non-IBC; $p < 0.001$) and the HER2-enriched subtype (22% in IBC vs. 9% in non-IBC; $p < 0.001$). Overall, 75% of the IBC samples were classified under the classically more aggressive subtypes, basal-like, HER2-enriched, claudin-low, or luminal B subtypes, whereas these subtypes account for 54% of the non-IBC samples. The number of genes with a uniquely IBC-specific gene expression profile represented only 3% of the global expression differences. Similar results were observed when triple negative breast cancer (TNBC) IBC samples (n=39) were compared with TNBC, non-IBC (n=49) [32]. No unique IBC-specific subtypes were identified by mRNA gene-expression profiling of those tumors. Nonetheless the limited number of genes assessed by the PAM50 platform could account for failure to observe specific genomic signature in IBC samples.

Alternative techniques to gene expression assays were also implemented in order to classify IBC. Among them are epigenetic analyses such as evaluation DNA methylation pattern and also gene copy number imbalances, both of which may capture genomic signatures which account for differential tumor behavior [33, 34]. For instance, Van der Auwera et al. reported results of methylation profiling on a cohort of IBC (n = 19) and non-IBC (n = 43) samples using the Illumina Infinium platform [35]. Methylation assay comparison of IBC with non-IBC led to the identification of only four differentially methylated genes (*TJP3*, *MOGAT2*, *NTSR2* and *AGT*). Mutational landscape imbalances of 49 IBCs and 124 non-IBCs were determined using high-resolution array-comparative genomic hybridization [36]. Genomic landscapes were overall similar when comparing the two groups. Differences were however appreciated, such as the more frequent gain of 1q, 8q and 17q regions in IBCs, or the more frequent loss of 4p, 8p, 11q, and 16q regions in non-IBCs. The median percentage of probe sets displaying a copy number aberrations for a sample was numerically higher in IBCs (3.7%, range 0.01–14%) than in non-IBCs (1.9%, range 0.01–26%; p = 6.1), even if a great variability between samples existed for both types, suggesting that the genome of IBC is more unstable.

Most recently, Hamm et al. used a custom hybridization capture-based probe library using Agilent SureDesign portal (Agilent Technologies) to analyze 20 primary IBC specimens [37]. This panel captured full coding regions of 208 cancer relevant genes and introns of 13 genes to detect the substitution, deletions, copy number changes in the 208 targeted genes and structural rearrangements in 17 genes. Analysis of the types of genetic variants revealed that missense mutations were

the most common variant (73%), followed by frameshifts (8%), splice site alterations (6%), nonsense mutations (5.5%), amplifications (5.5%), and in-frame insertions-deletions (3%). In total, NGS identified 391 genetic variants in 19 IBC tissues. The 5 most commonly altered genes were: *TP53* (58%), *HER2* (53%, all amplifications), *ATM* (53%), *APC* (37%), and *HER3* (26%).

In comparison with the mutational profile of a large non-IBC cohort (825 samples) in the TCGA, mutations in the p53 and *PIK3CA* genes are equally common when compared to the IBC mutational landscape [27]. *FGFR* and *HER2* aberrations seem to occur at similar frequency in non-IBC when compared to IBC, whereas *PIK3CA* mutations are more common in non-IBC (38%), probably because frequently associated with luminal disease. Moreover, *PTEN* abnormalities are less common in non-IBC (3%) [27, 38].

Qualitative mutational landscapes have also been performed in larger series in recent years. Ross et al. reported a comprehensive genomic profiling on 53 IBC, formalin-fixed, paraffin-embedded specimens using the hybrid capture-based, FoundationOne™ assay [39]. The 53 sequenced IBC cases harbored a total of 266 genomic alterations (GA) with an average of 5.0 GA/tumor (range 1–15). The most frequently altered genes were *TP53* (62%), *MYC* (32%), *PIK3CA* (28%), *HER2* (26%), *FGFR1* (17%), *BRCA2* (15%), and *PTEN* (15%). In the TNBC subset of IBC, 8/19 (42%) showed *MYC* amplification (median copy number 8X, range 7–20) as compared to 9/32 (28%) in non-TNBC IBC (median copy number 7X, range 6–21). Although at 32%, the *MYC* amplification in IBC appeared to represent enrichment in this tumor type, comparison with the 24% *MYC* amplification rate in the non-IBC breast cancers did not reach statistical significance ($p = 0.26$) [39]. While these small studies offer some insights in novel molecular differences, larger studies are needed to confirm and validate differences. Furthermore, non-genomic molecular characterization may be able to explain the phenotypic discordance between IBC and non-IBCs, such exploring the epigenome, metabolome, microenvironment, and immunogenic characterization.

TRANSMEMBRANE GROWTH FACTOR RECEPTORS

HER2

The *HER2* gene encodes a transmembrane tyrosine kinase receptor that belongs to the epithelial growth factor receptor (EGFR) family. This family of receptors includes four members (EGFR/HER1, HER2, HER3 and HER4) that function by stimulating growth factor signaling pathways such as the PI3K/AKT/mTOR pathway [18]. Activation of receptor kinase function occurs predominantly via ligand-mediated hetero- or homo-dimerization. In the case of HER2, activation is also thought to occur in a ligand-independent manner, particularly when the receptor is found to be mutated or overexpressed [40]. Targeted strategies against the HER family have been developed in the realm of breast cancer. For instance the humanized monoclonal antibodies (e.g.: trastuzumab) prevent the dimerization of HER2 with other HER receptors. Pertuzumab in particular inhibits the pairing of the most potent signaling heterodimer, HER2/HER3, thereby providing a potent strategy for dual HER2 inhibition [41]. Furthermore small molecule tyrosine kinase inhibitors such as lapatinib have the ability to inhibit the kinase activity of these HER receptors opposing further cancer cell proliferation and survival [42].

A subgroup analysis of 62 patients with HER2+ IBC enrolled in a large phase 3 trial (NOAH trial) reported a 54.8% in breast pCR to neoadjuvant trastuzumab in combination with chemotherapy, versus 19.3% for patients receiving only chemotherapy ($p = 0.004$) [43]. In this study 235 patients with HER2+ locally advanced non-IBC or IBC were randomized to treatment with or without neoadjuvant and adjuvant trastuzumab [44]. Neoadjuvant chemotherapy consisted of doxorubicin 60 mg/m^2 plus paclitaxel 150 mg/m^2, every 3 weeks for three cycles, followed by paclitaxel 175 mg/m^2 administered every 3 weeks for four cycles. Cyclophosphamide (600 mg/m^2), methotrexate (40 mg/m^2), and fluorouracil (600 mg/m^2) were then given on days 1 and 8 every 4 weeks for three cycles. In the overall study population chemotherapy combined with trastuzumab significantly improved in breast pCR rate to 43% and event-free survival (EFS) in patients with HER2+ breast cancer hazard ratio of 0.59, 95%CI 0.38-0.90; $p = 0.013$. After a median follow-up of 5.4 years the EFS benefit derived from trastuzumab treatment is maintained with a 5-year EFS rate of 58% (95% CI 48-66) in this patient population [45].

In the NeoSphere trial patients with HER2+ IBC were enrolled into the study but represented less than 10% of the overall study population [46]. Patients treated pertuzumab and trastuzumab plus docetaxel had a significantly improved pCR rate 45.8% [95% CI 36.1–55.7] compared with those given trastuzumab plus docetaxel; 29.0% [20.6–38.5]; $p = 0.0141$). Likewise in the Tryphaena phase 2 trial, among the 225 patients with HER2+ breast cancer treated with the combination of pertuzumab, trastuzumab and chemotherapy in the neoadjuvant setting only 13 had HER2+ IBC [47]. pCR rates defined as the absence of invasive carcinoma in the breast ranged from 61-66% among patients treated with dual HER2 targeted and chemotherapy.

Kaufman et al. reported results of 126 patients with relapsed or refractory HER2+ IBC who were treated with lapatinib 1500 mg once daily in a non-randomized, open-label, phase 2 study [48]. Seventy five percent of the patients had been treated with at least one line trastuzumab-based regimen. No patients

achieved a complete response. The objective response rate by clinically evaluable skin-disease criteria was 40% but the objective response by rate by RECIST was only 15% (95% CI 9-24), which included only patients with metastatic or locally advanced, measurable disease. Median PFS was 14.6 weeks (95% CI 12.1–16.0), with median duration of response of 20.9 weeks (12.7–32.1). Likelihood of response to lapatinib was not affected by previous treatment with trastuzumab. A total of 45 (32%) study participants had serious adverse events, the most common ones were dyspnea (eight patients) and pleural effusion (six). Five patients had fatal adverse events that were possibly treatment related.

EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR)

EGFR is over-expressed in approximately 30% of patients with IBC and correlates with poor outcome [21, 22]. Of note EGFR has been implicated in IBC cell survival and metastasis *in vitro* and *in vivo* [49–51]. SUM149 pre-clinical cell models showed significant sensitivity to sensitivity to inhibition of EGFR and other members of the HER family [49, 52, 53]. Lapatinib is not only a potent inhibitor of the HER2 but also inhibits EGFR tyrosine kinase domains and has shown significant clinical efficacy in non-IBC HER2+ breast cancer [54, 55].

In a phase 2 single arm trial 49 patients with IBC were stratified according HER2 and EGFR expression in two different groups: cohort A (HER2+ plus or minus EGFR+ IBC) or cohort B (HER2- and EGFR+) [56]. Patients received lapatinib at 1,500 mg/d for 14 days, then lapatinib at 1,500 mg/d plus weekly paclitaxel (80 mg/m^2) for 12 weeks, followed by surgical resection or additional chemotherapy. pCR occurred in 18.2% (95% CI, 5.2% to 40.3%) of cohort A patients. Combined clinical response rate was 78.6% (95% CI, 63.2% to 89.7%) in cohort A patients. Cohort B was terminated because of slow accrual and lack of efficacy observed in IBC patients with HER2-/EGFR+ tumors enrolled onto a parallel study, EGF103009 [57]. These results indicate modest activity of lapatinib in patients with HER2+ IBC. Matsuda et al. recently reported preliminary results of phase 2 trial of neoadjuvant treatment with 4 cycles of the combination of anti-EGFR monoclonal antibody panitumumab (2.5 mg/kg) combined with nab-paclitaxel (100 mg/m^2), and carboplatin (AUC 2) weekly followed by 4 cycles of FEC (5-fluorouracil, 500 mg/m^2; epirubicin, 100 mg/m^2; cyclophosphamide, 500 mg/m^2) [58]. A total of 35 patients with HER2- IBC were treated and 7 of the 16 patients with triple negative IBC achieved a pCR (44%; 95% CI: 0.20-0.70). Retrospective data support that pCR rates of < 25% are generally obtained among patients with IBC treated with and anthracycline combined a taxane in the neoadjuvant setting [59].

These results indicate that EGFR could be a valuable target for treatment development in IBC. Targeting of multiple molecular aberrations may be one the promising strategies. For instance, preliminary evidence also supports that c-MET is overexpressed in IBC [60]. Further studies are needed to evaluate the potential role of c-MET inhibition in IBC.

mTOR/AKT PATHWAY

Aberrations in the PIK3/AKT/mTOR pathway are among the most common genomic abnormalities in breast cancer and are observed across in all subtypes of the disease [27]. In the metastatic setting mTOR inhibitor everolimus has shown significant clinical efficacy in combination with aromatase inhibitor and is now commonly used treatment option for patients with hormonal receptor, HER2- metastatic breast cancer progressing on anti-estrogen therapy [61]. *AKT* gene is not frequently mutated in breast cancer [62, 63]. However its activation by upstream molecules such as PIK3CA is a common phenomenon in breast tumors across different subtypes, which leads to activation of multiple AKT substrates controlling tumor growth and apoptosis [64, 65].

Immunohistochemical analysis of 45 cases of IBC showed over-expression of phosphorylated mTOR in approximately 90% of the cases [66]. Of note all tumor tissues were obtained from mastectomy specimens after treatment with anthracycline and or taxanes. In addition, patients with invasive, previously treated non-IBC also showed equally elevated rates of mTOR activation. Ross et al. recently reported the results of a cross-sectional study involving patients with metastatic IBC that had a diagnostic biopsy of primary tumor or metastatic lesion and subsequent NGS by Foundation One™ panel [39]. The analysis of 53 IBC tumor samples revealed a total of 266 genomic alterations were observed. Of note, abnormalities in the PI3K/AKT/mTOR pathway were seen in up 65% of the samples analyzed and 41 patients (77%) had HER2- disease. Furthermore, Hamm et al. evaluated 19 patients with primary and metastatic IBC using a targeted NGS panel that covered whole coding regions of 208 of the most common cancer related genes (copy numbers and somatic mutations) and rearrangements in 17 well characterized cancer genes [37]. Activity of the PI3K/AKT/mTOR pathway was further confirmed by immunohistochemistry for phosphorylated S6 in 95% (n = 18) of cases, a target of mTOR kinase activity.

A recent small cross-sectional study of 12 patients with diagnosis of HER2+ IBC who had tumor tissue biopsy upon progression on HER2 targeted therapy evaluated HER1-3 through reverse-phase protein microarrays (RPMA) assay analysis [67]. Phosphorylation of HER1-3 downstream signaling pathways such as JAK2,

AKT/mTOR and MEK1/2 were analyzed. Interestingly, 83% had mTOR activation, and most of these patients also had accumulation of its downstream proteins, S6 ribosomal protein and 4E-BP-1. In addition, 78% of patients with HER2 activation also had mTOR activation indicating that the AKT/PIK3/mTOR pathway could be a mechanism of resistance to HER2 targeted therapies in IBC.

The mTORC1 inhibitor rapamycin and everolimus showed only modest inhibitory activity of IBC HER2+ and HER2- pre-clinical cell models (SUM190 and SUM149 respectively) when compared with targeted agents such as lapatinib, sorafenib, and sunitinib indicating that further understanding of the PIK3/AKT/mTOR is needed in IBC [68, 69]. In parallel, in non-IBC HER2+ tumor mTOR inhibition with everolimus combined with trastuzumab only lead to modest improvement in PFS when compared to placebo [70]. In summary, future studies are needed to investigate the potential clinical impact of the PIK3/AKT/mTOR inhibition in IBC.

ANGIOGENESIS

The vascular endothelial growth factor (VEGF) is an important angiogenic mediator in breast cancer [71]. VEGF-A is a multifunctional cytokine widely expressed by tumor cells that acts through receptors (VEGFR-1, VEGFR-2, and neuropilin) expressed on vascular endothelium and on some other cells. It increases microvascular permeability, induces endothelial cell migration and division, reprograms gene expression, promotes endothelial cell survival, prevents senescence, and induces angiogenesis [72]. VEGF targeted therapy with the monoclonal antibody against VEGF-A, bevacizumab; seem to have modest clinical efficacy in patients with metastatic breast cancer [73, 74]. IBC is known to have high rates of endothelial cell proliferation and vascular density when compared to non-IBC, which could suggest potentially greater sensitivity to antiangiogenic therapies [75]. Wedam et al. reported results of a small cohort of 21 patients with both IBC (n=20) and (n=1) non-IBC treated with bevacizumab for cycle 1 (15 mg/kg on day 1) followed by six cycles of bevacizumab with doxorubicin (50 mg/m^2) and docetaxel (75 mg/m^2) every 3 weeks in the neoadjuvant setting [76]. Tumor biopsies were collected on cycles 1, 4, and 7; a median decrease of 66.7% in phosphorylated VEGFR2 in tumor cells and median increase of 128.9% in tumor apoptosis were seen after bevacizumab alone. These changes persisted with the addition of chemotherapy. One patient had a complete pathological had a complete pCR and 14 have partial clinical response overall response rate (ORR) of 67% (95% CI, 43% to 85.4%). The ORR results are comparable with historical controls treated with anthracycline/taxane combination without bevacizumab in the neoadjuvant setting (ORR 81%) [77].

The BEVERLY-1 (UCBG-0802) trial was a phase 2, single-arm trial, in which women with non-metastatic HER2- IBC were treated with neoadjuvant intravenous fluorouracil (500 mg/m^2), epirubicin (100 mg/m^2), cyclophosphamide (500 mg/m^2), and bevacizumab (15 mg/kg) during cycles 1–4 (21 days-cycle), then docetaxel (100 mg/m^2) and bevacizumab during cycles 5–8 [78]. After surgery, patients received adjuvant intravenous bevacizumab. After neoadjuvant therapy, only 19 of the 100 patients evaluable for efficacy analysis achieved a pCR. The most frequent grade 3–4 events during the neoadjuvant phase were neutropenia (89%), febrile neutropenia (37%), and mucositis 23%) and during the adjuvant phase the most frequent grade 3–4 adverse event was proteinuria (7%). One (1%) patient died of thrombotic microangiopathy after cycle 1, which was thought to be related to bevacizumab. Two patients (3%) developed transitory heart failure.

In the BEVERLY-2 study 52 patients with HER2+ IBC were treated with fluorouracil, epirubicin, cyclophosphamide, and bevacizumab (cycles 1–4) and docetaxel, trastuzumab, and bevacizumab (cycles 5–8) before surgery, followed by trastuzumab and bevacizumab for 30 weeks after surgery [80]. After neoadjuvant therapy, 33 of 52 patients had a pCR according to central review (63.5%, 95% CI 49.4–77.5). The most common adverse events were asthenia and nausea (both occurred in 36 [69%] of 52 patients). Only one grade 3 or worse adverse event regarded as related to bevacizumab was reported (hypertension, one patient). It is important to note that the pCR rates of this HER2+ IBC cohort was comparable to previously published studies, which did not add bevacizumab to the neoadjuvant treatment regimen of patient IBC [81]. Taken together these results indicate that the addition of bevacizumab to chemotherapy does not significantly improve pathological complete response rate in IBC.

One could hypothesize that higher expression of several non-VEGF angiogenic, lymphangiogenic, and vasculogenic factors could make blockade of VEGF by bevacizumab insufficient [7]. Indeed, pre-clinical data support that multityrosine kinase inhibitors such as sorafenib and sunitinib exert inhibitory activity against SUM149 IBC cell models [68]. For instance, pazopanib is an oral angiogenesis inhibitor targeting VEGF receptors 1-3, platelet-derived growth factor receptors-α/-β [82, 83]. Cristofanilli et al. reported results of a multi-center phase 2 study evaluating lapatinib, pazopanib, or the combination in patients with relapsed HER2+ inflammatory breast cancer [84]. In Cohort 1, 76 patients were randomized 1:1 to receive lapatinib 1,500 mg plus placebo or lapatinib 1,500 mg plus pazopanib 800 mg (double-blind) once daily until disease progression, unacceptable toxicity, or death. Due to high-grade diarrhea observed with this dose combination in another study (VEG20007), Cohort 1 was closed. The protocol was amended such that an additional

88 patients (Cohort 2) were randomized in a 5:5:2 ratio to receive daily monotherapy lapatinib 1,500 mg, lapatinib 1,000 mg plus pazopanib 400 mg, or pazopanib 800 mg monotherapy, respectively. The primary endpoint was ORR and secondary endpoints included duration of response, progression-free survival (PFS), overall survival, and safety. In Cohort 1, ORR for the lapatinib (n = 38) and combination (n = 38) arms was 29% and 45%, respectively; median PFS was 16.1 and 14.3 weeks, respectively. Grade 3 adverse events were more frequent in the combination arm (71%) than in the lapatinib arm (24%). Dose reductions and interruptions due to AEs were also more frequent in the combination arm (45 and 53%, respectively) than in the lapatinib monotherapy arm (0 and 11%, respectively). In Cohort 2, ORR for patients treated with lapatinib (n = 36), lapatinib plus pazopanib (n = 38), and pazopanib (n = 13) was 47, 58, and 31%, respectively; median PFS was 16.0, 16.0, and 11.4 weeks, respectively. In the lapatinib, combination, and pazopanib therapy arms, grade ≥3 AEs were reported for 17, 50, and 46% of patients, respectively. The lapatinib-pazopanib combination was associated with a numerically higher ORR but no increase in PFS compared to lapatinib alone, which was a secondary endpoint of the study. The combination also had increased toxicity resulting in more dose reductions, modifications, and treatment delays. As novel agents are developed that are better tolerated, the improve response rates in this study may suggest a role for multi-target angiogenesis agents in IBC.

JANUS KINASE/SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION (JAK/STAT) PATHWAY

JAK kinases are activated through tyrosine phosphorylation of the cytoplasmic domains of cytokine receptors upon cytokine binding. JAK2 activation promotes recruitment to the receptor complex of the transcription factors STAT3 and STAT5 [85]. JAK2-mediated STAT phosphorylation leads to the formation of stable homodimers and heterodimers, which leads to their nuclear translocation. Once in the nucleus, STAT molecules bind specific promoter DNA sequences that result in the transcription of genes that regulate cell proliferation, differentiation, and apoptosis (e.g., Bcl-xL, cyclin D1, and PIM1) [85, 86]. STAT3 has been implicated in many aspects of tumorigenesis, including differentiation, proliferation, apoptosis, increased sensitivity to cytotoxic agents, angiogenesis, recruitment of immune cells, and metastasis [87]. Evidence supports that, in breast cancer, JAK2 activates STAT3 and is found to be significantly activated when compared to non-neoplastic breast tissue [88, 89]. In one series of 45 samples of previously treated IBC samples immunohistochemical analysis showed activated JAK2 (pJAK2) levels were similar between IBC (95.2%, 1+ or 2+) and treated IDC (91.7%, 1+ or 2+; 4.2%, 3+), untreated IDC had lower levels (80.0%, 0; 20.0%, 1+) ($p < 0.0001$). For pSTAT3, 55.0% of IBC tumors had 1+ or 2+ levels with 45% of tumors having level 0. In treated IDC, 62.5% had 1+ or 2+ expression with 37.5% of tumors having level 0. This is in contrast to untreated IDC where 92.3% of tumors had 0 level ($p = 0.0001$) [66]. These results indicate that JAK/STAT pathway activation could lead to treatment resistance in IBC. A phase 1/2 study of combination ruxolitinib (kinase inhibitor of JAK/STAT) with neoadjuvant chemotherapy for triple negative IBC is ongoing (NCT02041429).

CELL CYCLE CONTROL PATHWAYS

MYC oncoprotein interacts closely with the cell cycle machinery in depriving the cell of the normal control of progression through the G_1 phase of the cell cycle into the S phase. MYC protein acting with its Max partner is able to induce expression of the growth-promoting cyclin D2 and CDK4 (Figure 1) [90, 91]. At the same time MYC can promote degradation of $p27^{Kip1}$ CDK inhibitor as well as E2F1 favoring advancement into the S phase [92, 93].

Ross et al. reported the results of genomic sequencing of 53 IBC samples from the primary or a metastatic site [94]. Alterations in cell cycle regulatory genes were identified as follows: *MYC* (31%), *CCND1* (9%), *RB1* alterations (9%), and *CDKN2A* (8%). All *MYC* aberrations consisted of amplifications. Of the 19 TNBC IBC cases 8 (42%) had *MYC* amplification, whereas only 9 among 36 non-TNBC IBC cases harbored *MYC* amplification in 9 (25%). Indeed, the luminal, estrogen receptor- positive, IBC cell line (SUM190) exhibited significant sensitivity to CDK4/6 inhibitor (palbociclib) when compared to other solid tumors [95]. While a standard of care in conjunction with anti-hormone therapy in ER+ MBC, the clinical efficacy of palbociclib has not been reported in IBC.

RHOC GTPASE

In a previous series of studies, RhoC GTPase overexpression has been identified in >90% of IBCs and defined *RhoC* as a mammary oncogene involved in conferring the metastatic phenotype in IBC and estrogen receptor negative non-IBC [96–98]. Protein farnesyl transferase inhibitors revert the RhoC GTPase-induced inflammatory breast cancer phenotype [99]. Tipifarnib is an oral protein farnesyl transferase inhibitor. Despite the pre-clinical rationale, in a phase 2 trial of 22 patients with HER2- IBC, only one patient achieved a pCR after treatment with tipifarnib and conventional anthracycline/taxane-based neoadjuvant chemotherapy indicating lack of single activity of tipifarnib [100]. One potential strategy for future drug development could be to combine

farnesyltranferase enzyme inhibitors with STAT3 targeted therapies in IBC and there also seems to be interaction between these two pathways in non-IBC [101]. Furthermore, IBC pre-clinical models support that RhoC GTPase is a substrate for Akt1 and its phosphorylation is absolutely essential for IBC cell invasion [102]. Future drug development should take into account potential interactions between RhoC GTPase and the PIK3/AKT/mTOR pathway.

ANAPLASTIC LYMPHOMA KINASE (ALK)

EML4/ALK fusion is present in about 5% of patients with non-small cell lung cancer (NSCLC) [103]. The presence of EML4/ALK translocation is predictive of clinically meaningful benefit from treatment with the oral tyrosine kinase inhibitor crizotinib and alectinib in patients with NSCLC [104, 105]; ALK aberrations are also thought be rare in non-IBC (<1%) [106]. Analysis of 25 samples of IBC for ALK genetic abnormalities was performed. These studies revealed that 20/25 (80%) had some form of ALK aberration (i.e.: increased copy number, low level ALK gene amplification, or ALK gene expression), with an increased prevalence of ALK alterations in basal-like IBC. One of 25 patients was identified as having an *EML4-ALK* translocation [107]. A recent preliminary report indicated that ALK protein over-expression is not thought to be a feature of IBC [108]. The predictive value of *ALK* genomic aberrations is being assessed in an ongoing phase 1 trial in which patients with solid tumors including IBC, will be treated with LDK378 (selective inhibitor of ALK) (NCT01283516).

EPIGENETIC MODULATION

Polycomb group (PcG) protein enhancer of Zeste homolog 2 (EZH2) is among the DNA methylation modulation mechanisms, which interacts-within the context of the PcG repressive complexes 2 and 3 (PRC2/3)-with DNA methyltransferases (DNMTs) and associates with DNMT activity *in vivo*. Binding of DNMTs to several EZH2-repressed genes depends on the presence of EZH2 [109]. EZH2 is frequently expressed (~75%) in human IBC and its expression correlates with worse clinical outcome [110]. EZH2 is expressed at higher

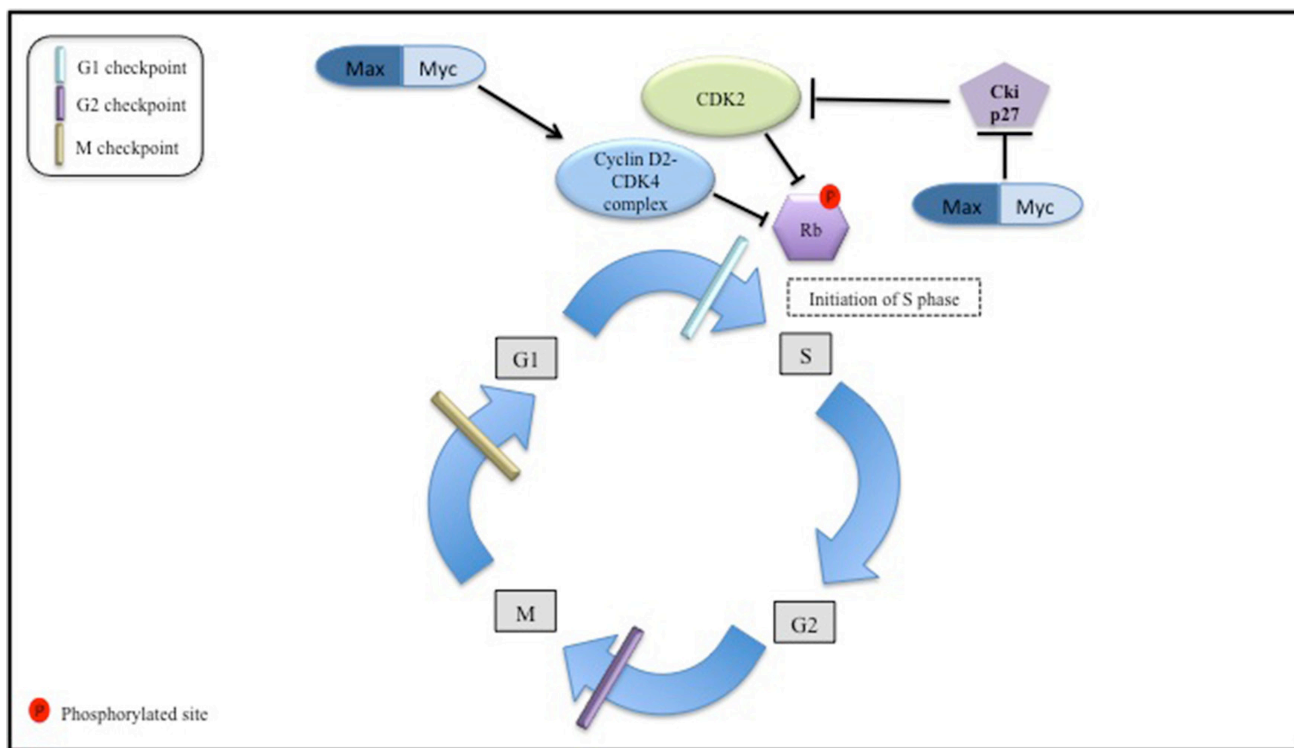

Figure 1: The Myc interaction with the cell cycle machinery. this figure is a simplified depiction of the role Myc oncoprotein in regulation of the cell cycle machinery. The normal cell replication processes are represented i.e., G1 first growth period to S DNA replication phase, G2 second growth period, and M, which is the mitosis period. Cyclin D2-CDK4 and CDK2 inactivate retinoblastoma protein (Rb) through phosphorylation. The latter event allows for cell cycle progression from G1 to S phase. Max/Myc complex targets cyclinD2-CDK4 complex formation ultimately stimulating cell cycle progression. Also cycle dependent kinase 2 (CDK2) complex activation is depicted through the abrogation of inhibitory action of Cki27 by active Max/Myc complex. When active CDK2 complex promotes initiation of S phase.

levels in human IBC cell lines compared with normal human mammary epithelial cells, and the knockdown of EZH2 expression significantly suppressed cell growth and tumor spheroid formation of human IBC cells *in vitro*. In addition, EZH2 knockdown inhibited the migration and invasion of IBC cells. Significantly, EZH2 knockdown suppressed the angiogenesis and tumor growth of IBC cells *in vivo* [111]. Furthermore pre-clinical evidence supports that epigenic modulation through histone deacetylase inhibitors (HDAC) may be promising in IBC models. Robertson et al. reported results of *in vivo* and *in vitro* IBC models supporting antitumor activity of the HDAC inhibitor romidepsin [112]. Not only did HDAC inhibition cause lower levels of VEGF-A and hypoxia-induced factor 1α in a xenograft model but it also blocked self-renewal/clonogenicity of tumor spheroids. In addition, romidepsin alone effectively inhibited SUM149 primary tumor growth and was synergistic with paclitaxel in blocking development of SUM149 metastatic lesions at multiple sites. Preliminary results from a phase 1 trial which tested the combination of romidepsin with nab-paclitaxel among 9 patients with metastatic, refractory HER2- IBC showed one complete response and two disease stabilizations [113]. Toxicities related to romidepsin included neutropenia, anemia and fatigue. Ongoing studies are evaluating HDAC inhibitors alone or in combination with targeted therapies [114, 115].

IMMUNE CHECKPOINT INHIBITORS

Immunotherapy with checkpoint inhibitors has made a significant impact in the treatment of melanoma, renal cell carcinoma and NSCLC in recent years [116–119]. New agents such as nivolumab and pembrolizumab [a fully human IgG4 programmed death 1 (PD-1) immune-checkpoint inhibitor antibody] selectively blocks the interaction of the PD-1 receptor with its two known programmed death ligands, PD-L1 and PD-L2, disrupting the negative signal that regulates T-cell activation and proliferation [120]. There is preliminary evidence of positive correlation between high mutational burden of tumors and clinical benefit from immunotherapy strategies (i.e. checkpoint inhibitors anti-CTLA-4 and anti-PD-1 antibodies), with remarkable effects seen with tumors displaying the highest rates of mutations such as melanoma [121, 122]. This is also illustrated by the anti-tumoral immunologic response to anti-PD-1 antibody in patients with colorectal cancer and increased mutational burden secondary to mismatch repair deficiency [123]. Nonetheless lack of definition and standardization of measures of tumor mutational load, and prospective validation of its predictive value indicate that better understanding of biomarkers predictive of benefit from immune checkpoint inhibition remains an unmet need.

The potential importance of immune checkpoint-guided therapy in breast cancer is underscored by recent report of PD-1 inhibitor activity in triple negative non-IBC. Pembrolizumab, which is a monoclonal anti-PD-1 antibody was tested in a phase 1b trial on 32 female patients with PD-L1 immunohistochemistry (IHC) + and heavily pretreated metastatic recurrent triple negative non-IBC. The disease control rate (i.e., percentage of patients with best response of complete response, partial response, or stable disease for ≥ 24 weeks) was 25.9% (95% CI, 11.1% to 46.3%) [124]. Avelumab, an anti-PD-L1 IgG1 antibody, showed modest anti-tumor activity among 57 patients with triple negative non-IBC with only 5 partial responses observed (8.8%; 95% CI: 2.9, 19.3) [125]. In patients with triple negative non-IBC who had PD-L1+ immune cells within the tumor, 44.4% (4 of 9) had partial responses, compared with 2.6% (1 of 39) for triple negative non-IBC and PD-L1– immune cells.

The role of immune infiltrate and immune checkpoints was also investigated in relation with genomic abnormalities in IBC samples [37]. The pathological examination of 20 IBC tissue samples identified a subset of IBC tumors associated with infiltration of immune cells. IHC staining identified the majority of infiltrating cell populations as CD8+ cytotoxic T cells and high levels of CD8+ infiltration were observed in 5/12 tumors. In order to explore the possible role of PD-L1 in IBC, the investigators performed IHC staining of IBC tissues. Evaluation of PD-L1 staining demonstrated low-intensity tumor cell staining in 3/12 tumors studied and high-intensity tumor cell staining in 1/12 tumors. PD-L1 mRNA expression has been reported to by as high as 38% among patients with IBC, which is higher that non-IBC (28%) and correlates positively with pCR [126].

Notably, somatic mutation rates were significantly higher in high infiltration *vs.* low infiltration tumors ($p<0.05$) [37]. The authors speculated that this correlation between somatic mutation rate and immune cell infiltration might be related to the exposure of tumor neo-antigens to the immune system. A phase 2 clinical trial for patients with metastatic IBC assessing the efficacy of anti-PD-1 inhibitor monoclonal antibody (pembrolizumab) is under development (NCT02411656) (Table 1).

DISCUSSION

IBC is rare but aggressive disease, in which improvements in therapeutic strategies are urgently needed. Its presentation, aggressive clinical course, and frequent distant recurrence indicate that IBC is in fact a distinct clinical biological entity rather than a subtype on the spectrum of locally advanced breast cancer. Key molecular differences include significant alterations in the PI3K and JAK/STAT pathways, elevated aberrations in DNA-repair genes and cell-cycle regulations suggesting of significant genomic instability contributing to treatment resistance. Furthermore higher rates of HER2 overexpression/amplification are seen

Table 1: Drugs under development for IBC – trials accrue patients with IBC only

Agent (s)	IBC subtype	Phase of study	Mechanism of action targeted therapy	Clinicaltrial.gov Identification number
Ruxolitinib	TN	2	JAK/STAT TKI	NCT02041429
Paclitaxel/trastuzumab/pertuzumab	HER2 +	2	Anti-HER2 mAb	NCT01796197
Afatinib	HER2 +	2	EGFR and HER2 TKI	NCT01325428
Nintedanib	HER2 -	2	Multi TKI	NCT02389764
Dovitinib	HER2 -	2	Multi TKI	NCT01262027
Panitumumab/nab-paclitaxel/ carboplatin	HER2 -	2	Anti-EGFR mAb	NCT01036087
Eribulin/doxorubicin/ cyclophosphamide	HER2 -	2	–	NCT02623972
Pembrolizumab	NS	2	Anti-PD-1 mAb	NCT02411656
SU5416/doxorubicin	NS	1	VEGFR TKI	NCT00005822
Nab-paclitaxel/gemcitabine/ epirubicin	NS	2	–	NCT00193206
Bevacizumab/cyclophosphamide/ 5-FU/epirubicin	NS	1	Anti-VEGF mAb	NCT01880385
Bevacizumab	NS	2	Anti-VEGF mAb	NCT00016549
Docetaxel/5-FU	NS	3	–	NCT02324088

Accessed on July 18th 2016 at www.clinicaltrials.gov
Abbreviations: Epithelial growth factor receptor (EGFR), fluorouracil (5-FU), inflammatory breast cancer (IBC), janus kinase/ signal transducers and activators of transcription (JAK/STAT), monoclonal anti-body (mAb), receptor tyrosine-protein kinase erbB-2 (HER2), triple negative (TN), tyrosine kinase inhibitor (TKI), vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor (VEGFR).

in IBC and neoadjuvant treatment with trastuzumab resulted in improved outcomes for patients with HER2+ IBC [43]. There is no prospective trial appropriately powered to assess the efficacy of neoadjuvant treatment with chemotherapy combined with pertuzumab and trastuzumab in HER2+ IBC. Nonetheless based on improved pCR rates seen in non-IBC these agents are recommended by current guidelines for the neoadjuvant treatment of HER2+ IBC.

As with other rare or under diagnosed diseases the evidence for the treatment of IBC stems from small prospective trials and subgroup analysis of larger non-IBC prospective studies. As IBC has no histological diagnostic criteria, every effort must be made to accomplish international standardization of diagnosis and treatment and facilitate future research [5, 127]. Research groups have also initiated prospective biorepository studies in order to facilitate future tumor tissue and blood-based biomarker studies in IBC (NCT00477100, NCT00646555, NCT00340158). Efforts are increasing to improve the biological understanding of IBC and to conduct clinical trials, which are specific for patients with IBC (Table 1). Nonetheless in the ever-expanding field of biomarker-based research reliable correlations between pCR and tumor genomic aberrations remain to be determined in IBC, except for HER2 amplification [128].

Further understanding of molecular biology of IBC focusing on the tumor microenvironment and immunity may help explain the different clinical behaviors of IBC and non-IBC e.g. the role of mesenchymal transitional cells in promoting E-cadherin (pivotal to IBC metastasis) expression in IBC cell models and metastases in xenografts [50, 75].

CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest

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