Medical Research Archives. Volume 4 Issue 6

Targeting triple-negative breast cancer: optimizing therapeutic outcomes – UPDATE

Authors.
1. Wendie D den Brok,
2. Karen A Gelmon

Authors note

1. Breast Fellow, Department of Medical Oncology, BC Cancer Agency, Vancouver, Canada
Email:wdenbrok@bccancer.bc.ca

2. Medical Oncologist, Department of Medical Oncology, BC Cancer Agency, Vancouver, Canada
Email:kgelmon@bccancer.bc.ca

ABSTRACT

Background: Triple-negative breast cancer (TNBC) is an aggressive, heterogeneous clinical breast cancer subtype that currently lacks approved targeted therapies. Research aimed at understanding the mutational and transcriptional landscape of TNBC is being achieved through gene expression analysis and large-scale genomic projects, resulting in identification of potential drug targets.

Design: A review of PubMed and conference databases was carried out to identify randomized clinical trials in TNBC as well as early phase trials of emerging targeted therapies.

Results and Discussion: The role of platinums and poly(ADP-ribose) polymerase inhibitors continues to be a focus of clinical trials with attention now on developing a predictive biomarker that identifies "BRCA-like" tumours. The previously identified six TNBC subtypes has been revised to four and has provided further insight into the role of the androgen receptor and immune system, both of which are emerging as promising targets in select patients either as monotherapy or in combination with other immune therapies, chemotherapy or targeted therapy. Other novel targets include MET inhibition and signaling pathways such as the MAPK, PI3K and JAK/STAT pathways. Antibody-drug conjugates are also of interest. Targeting the angiogenesis and epidermal growth factor receptor pathways have had limited efficacy in the treatment of TNBC.

Conclusions: As researchers begin to understand the underlying biology of TNBC and identify predictive biomarkers, more select patient populations are being treated with targeted therapies, which is a promising step forward in filling the current void of approved targeted treatment options for patients with TNBC.

Key Words: androgen receptor, breast cancer, breast cancer molecular subtypes, cancer treatment, immune checkpoint blockade, targeted therapy, triple-negative breast cancer.

Introduction:

Triple-negative breast cancer (TNBC), defined by lack of estrogen receptor (ER), progesterone receptor (PR) and HER2-neu amplification, remains a difficult to treat and aggressive clinical subtype of breast cancer without approved targeted therapies. TNBC accounts for approximately 15% of all breast cancer diagnoses affecting a disproportionate number of young women compared to other breast cancer subtypes. Researchers are beginning to identify sub-classifications of this heterogeneous disease by focusing on the mutational and transcriptional landscapes of TNBC tumours. Understanding the underlying biology allows for the development of predictive biomarkers which in turn will potentially lead to the development of targeted therapies in select TNBC patient populations, a need that cannot be emphasized enough given the lack of success in developing targeted therapies in unselected TNBC patient populations.

Germline mutations in *BRCA1* and *BRCA2 (*herein called *BRCA)*, which occur in 20% of patients with TNBC,[1] represent the first real biomarkers in TNBC. The *BRCA* genes are involved in the homologous recombination (HR) pathway,[2] a high fidelity double-stranded DNA repair mechanism, which have informed further research around the role of HR in TNBC. It is now estimated that up to 40% of patients with TNBC have defects in the HR pathway,[3] either through *BRCA* germline mutation or via other mechanisms, and those without *BRCA* mutations (ie. *BRCA* wild type [WT]) are said to be "BRCA-like" or exhibit "BRCAness". The base excision repair (BER) pathway, involved in single-strand DNA repair, is another pathway that can be exploited in patients with HR defects via the process of synthetic lethality. Targeting these pathways in patients with *BRCA* germline mutations has been promising. Researchers are now focused on developing a biomarker to identify those patients who harbour "BRCAness", a subgroup also expected to benefit from drugs targeting these DNA repair pathways. Whether "BRCAness" will give similar responses to targeted therapies as tumours with germline *BRCA* mutations is not known.

The intrinsic subtypes of breast cancer as determined by PAM50 (luminal A, luminal B, HER2-enriched, and basal-like), defined the basal-like subtype, which overlaps with up to 80% of TNBC. Lehmann et al.[4] reported on gene expression analyses which initially sub-classified TNBC into 6 distinct molecular subtypes (basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL) and luminal androgen receptor (LAR) subtypes). These molecular subtypes of TNBC have recently been refined to four distinct

molecular subtypes (BL1, BL2, M, LAR) [5] since gene expression profiles of the IM and MSL molecular subtypes are now thought to reflect high expression of tumour infiltrating lymphocytes (TILs) and the surrounding stroma, respectively, and not distinct molecular subtypes. These researchers have retrospectively examined neoadjuvant chemotherapy responses from pretreatment biopsies of 300 TNBC patients that underwent subtyping using the TNBC subtype and PAM50. They found differential response to neoadjuvant chemotherapy based on the molecular subtype. These 4 TNBC subtypes and the presence of TILs may help identify patients who stand to benefit from cytotoxic chemotherapy, immunotherapy and other targeted therapies such as anti-androgens.

Large scale genomic projects and translational research have also uncovered other molecular characteristics in TNBC that show promise in early phase clinical trials including receptor targets and signaling pathways such as the well known MAPK and PI3K pathways but also other emerging pathways such as the JAK/STAT pathway.

This updated review on targeting TNBC identifies studies performed since the original article was published in 2012 and highlights progress in targeting select patient TNBC populations based on mutational and transcriptional analyses.

Methods

PubMed (through July 21 2016), the American Society of Clinical Oncology (2015 and 2016), and San Antonio Breast Cancer Symposium (2014 and 2015) databases were searched at the title and abstract levels using the search terms 'triple negative breast cancer' or 'TNBC' to identify studies investigating targeted therapy or platinum-based therapy in TNBC. ClinicalTrials.gov database was searched to identify ongoing clinical trials in TNBC.

Targeting deficiencies in DNA repair: platinum-base agents and poly(ADP-ribose) polymerase 1/2-inhibitors

BRCA germline mutations and defects of HR (ie. BRCAness) via other methods such as mutations in other HR associated genes, epigenetic changes such as *BRCA1* promotor methylation, copy number aberrations, or structural rearrangements affect approximately 40% of TNBC patients.[3] A number of studies have shown that patients deficient in HR derive greater benefit from DNA damaging therapies such as platinum salts and PARP inhibitors. Although *BRCA* germline mutations are easily identified using currently available methods, a biomarker to identify those who are *BRCA* WT but still have deficiencies in the HR pathway has been a difficult endeavor. Researchers continue to work on developing a robust biomarker of HR deficiency with the Myriad Genetics

myChoice® assay furthest along in terms of development and clinical utility.

Platinum-based chemotherapy

In the neoadjuvant setting, platinum agents combined with chemotherapy have resulted in higher rates of pathologic complete response (pCR) especially in *BRCA* germline mutated TNBC but also in a subset of *BRCA* WT. A number of phase 2 trials[6–7] have shown improved pCR rates with carboplatin in addition to various chemotherapies and targeted therapies in *BRCA* mutated tumours with pCR rates ranging from 45% to 67%. In the initial analysis of the GeparSixto trial[6] TNBC patients with stage II-III disease who received weekly paclitaxel, non-pegylated liposomal doxorubicin and bevacizumab plus weekly carboplatin (AUC 2, later reduced to AUC 1.5) had improved pCR rates, 84/158 (53%) versus 58/157 (37%) who did not receive carboplatin. In the updated analysis presented at SABCS in 2015, pCR rates remained higher and had better disease-free survival in the carboplatin-treated arm regardless of *BRCA* mutation status.[8]

The CALGB 40603 study[9] also showed improvements in pCR rates for 443 patients with stage II-III TNBC when treated with standard weekly paclitaxel followed by dose-dense doxorubicin/cyclophosphamide (AC) in addition to carboplatin+/- bevacizumab compared to those treated with standard chemotherapy. Those treated with the addition of carboplatin (AUC 6 every 3 weeks) had a statistically significant increase in pCR in both the breast and axilla compared to those who did not receive carboplatin (54% vs 41%, p=0.0029). At the 2015 San Antonio Breast Cancer Symposium updated results reported that pCR was associated with better 3-year event-free survival (74%) and 3-year overall survival (83%). This study did not report on *BRCA* mutation status but biomarker correlates of HR deficiency are a work in progress with the myChoice® assay to try to predict those tumours that may benefit from platinum-based regimens.[10,11]

In patients with advanced TNBC, objective response rates (ORR) with platinum agents have been shown to be superior to non-platinum chemotherapy for patients with *BRCA* mutated TNBC but not *BRCA* WT.[12] ORR was 68% in *BRCA* mutated TNBC treated with carboplatin compared to 28% in *BRCA* WT TNBC treated with carboplatin. The dichotomized myChoice® assay score failed to discriminate between patients who would be sensitive to carboplatin versus docetaxel; however, the assay was performed on archival tissue from the primary tumour suggesting that advanced TNBC may have different underlying biology compared to early stage TNBC.

PARP inhibition

A phase III clinical trial of iniparib[13] failed to show statistically significant differences in PFS and OS, but iniparib exhibits very weak PARP inhibition. There is now a resurgence of interest in the more potent and true PARP inhibitors for the treatment of breast cancer. In the advanced setting, olaparib showed good results in phase II clinical trials of BRCA mutated cancers but no responses in a small, unselected cohort of TNBC with *BRCA* WT. [14,15] Phase III clinical trials of olaparib in *BRCA* mutated, HER2 negative breast cancers are underway in the adjuvant, neoadjuvant and advanced settings.[16] Reports of the metastatic trial are expected soon and could change clinical practice.

The phase II adaptive randomization clinical trial, I-SPY 2, has evaluated veliparib plus carboplatin in addition to standard chemotherapy for patients receiving neoadjuvant chemotherapy. In the subgroup of patients with TNBC, pCR rates were estimated to be 51% in the PARP inhibitor/carboplatin plus chemotherapy arm versus 26% in the standard chemotherapy arm. A deleterious or suspected deleterious mutation in *BRCA* was found in 17% of the veliparib/carboplatin arm compared to 7% of the standard chemotherapy arm.[17] Although *BRCA* mutation status may have impacted rates of pCR, these results suggest that there is a subgroup of patients who are *BRCA* WT that stand to benefit from DNA damaging therapies. This adaptive clinical trial has incorporated two biomarkers of HR deficiency in an attempt to predict those who will benefit from veliparib/carboplatin.[18]
Cisplatin with or without low dose rucaparib has been studied after preoperative chemotherapy in patients with TNBC or *BRCA* germline mutations who had residual disease (lymph node involvement or >2 cm of invasive disease after standard neoadjuvant chemotherapy). The primary outcome of 2-year DFS was not different between the cisplatin/rucaparib arm versus the cisplatin arm (63.1% vs 58.3%; p=0.43) and *BRCA* mutation status did not impact 2-year DFS. However, the dose of rucaparib (24-30 mg IV on days 1-3 every 3 weeks x 4 followed by 30 mg IV or 100 mg orally weekly for 24 weeks) was substantially lower than the monotherapy dose used in phase II trials (600 mg orally twice daily) and may have resulted in inadequate PARP inhibition.[19]

Phase III trials of niraparib (NCT01905592) and talazoparib (NCT01945775) versus physician's choice in *BRCA* mutated advanced breast cancer are currently underway.

Immune checkpoint inhibition – PD-1/PD-L1 monoclonal antibodies

Immune checkpoint inhibitors targeting the T-cell inhibitory receptor programmed cell death 1 (PD-1) and its ligand, PD-L1, have significantly altered the treatment paradigm of various cancers including melanoma,

non-small cell lung cancer, renal cell cancer and bladder cancer. TNBC is associated with high levels of genomic instability and higher mutational burden which has been shown to be associated with benefit in other malignancies treated with immune checkpoint blockade therapies.[20,21] TNBC is also associated with higher levels of tumour-infiltrating lymphocytes (TILs) compared to other breast cancer subtypes, a finding that is associated with improved outcomes.[22] TILs are found in all TNBC molecular subtypes with the highest expression in BL2 (in the refined molecular TNBC subtype classification) and may be considered representative of the immune state of the tumour.[5] PD-L1 expression is measured on TILs but its role alone as a predictive biomarker has been inconsistent and continues to be investigated.

One of the first immune checkpoint inhibitors to be studied in TNBC is pembrolizumab, a PD-1 monoclonal antibody. In the phase Ib KEYNOTE-012 Study[23] 32 of 111 (29%) patients with advanced TNBC and positive for PD-L1 expression received pembolizumab IV at 10 mg/m2 every 2 weeks. In this heavily pretreated population (47% of patients had ≥ 3 lines of therapy in the metastatic setting), ORR was 18.5%. Because this study enrolled only those patients with PD-L1 expression, PD-L1 as a potential biomarker could not be assessed. Pembrolizumab given at 200 mg IV every 3 weeks is now in phase II and III clinical trials as monotherapy (NCT02447003, NCT02555657) and in combination with chemotherapy (NCT02819518).

Pembrolizumab is also being studied in early phase clinical trials for patients with metastatic TNBC in combination with the PARP inhibitor niraparib (NCT02657889), eribulin mesylate (NCT02513472) and carboplatin plus gemcitabine (NCT02755272).

Atezolizumab, a PD-L1 monoclonal antibody, resulted in ORR of 19% in a phase I study in patients with PD-L1 positive TNBC.[24] The combination of immune checkpoint inhibitors and cytotoxic chemotherapy may be synergistic by increasing cancer cell antigens (neoantigens);[25,26] a phase Ib clinical trial of atezolizumab (800 mg every 2 weeks) in combination with nab-paclitaxel (125 mg/m2 every week for 3 of 4 weeks) in patients with metastatic TNBC and ≤3 lines of therapy in the advanced setting resulted in ORR of 42%. Responses were seen regardless of PD-L1 expression.[27] Atezolizumab is now in phase III clinical trials as first-line therapy for patients with metastatic TNBC in combination with nab-paclitaxel versus nab-paclitaxel plus placebo with ongoing correlative biomarker assessment of PD-L1 (NCT02425891).

Novel immune checkpoint inhibitors are in early phase clinical trials of TNBC (NCT02484404, NCT01772004)

while other early phase clinical trials are focusing on dual immune checkpoint blockade (NCT01928394, NCT02554812) or the combination of immune checkpoint blockade with immune modulators and vaccines (NCT02658890, NCT02528357, NCT02018458). Results of a phase III trial of the vaccine NeuVax™ in patients with high risk, HER2-negative breast cancer after definitive standard local and systemic treatment are pending with 3-year DFS as the primary endpoint (NCT01479244).

Immune checkpoint inhibitors are generally well tolerated with fatigue, nausea, fever, and arthralgias/myalgias being the most commonly reported side effects in clinical trials. As well, a unique spectrum of immune related adverse events are associated with these drugs including colitis, pneumonitis, pancreatitis, dermatitis, hepatitis and endocrinopathies. Patients with pre-existing autoimmune diseases have been excluded from clinical trials and the risk of potentiating underlying autoimmune disease with the use of these drugs is not known. As well, many trials excluded patients with brain metastases, a common occurrence in TNBC.

Anti-androgens

The androgen receptor (AR) is expressed in approximately 60% of all breast cancer subtypes and associated with improved 3- and 5-year OS.[28] The expression of AR has differential effects on tumourigenesis in pre-clinical studies depending on ER/PR expression with AR expression thought to have stimulatory effects in TNBC and inhibitory effects in ER/PR positive breast cancers.[29] In TNBC, reports of AR expression are highly variable (6.6% to 75%) reflecting different methods of testing and cutoffs for positivity.[30] In the refined TNBC molecular subtypes described by Lehmann et al.,[5] 16% of tumours were found to be of the luminal androgen receptor (LAR) subtype that is biologically driven by the androgen receptor (AR). Patients with the LAR subtype had lower grade tumours, higher degree of nodal involvement at diagnosis, more advanced stage and age at diagnosis and a higher predilection to metastasize to bone compared to other TNBC subtypes. The LAR subtype has been associated with a lower pCR rate after neoadjuvant chemotherapy compared to the BL-1 subtype.[5] Although this subtype has been identified via gene expression analysis by other researcher groups[31, 32] and is emerging as an important target in a subset of TNBC patients, the best method for identifying patients who overexpress the AR (IHC versus gene expression signatures or both) is not clear.[33]
On PAM50 the LAR subtype is frequently associated with the HER2 enriched or the luminal subtypes.

Three phase II trials have reported outcomes in metastatic TNBC with anti-androgen therapy and all considered AR positivity as >10% nuclear staining of the AR by

immunohistochemistry (IHC).[34,35,37] The first by Gucalp et al.[34] reported outcomes in patients with TNBC treated with 150 mg of daily oral bicalutamide. Of 424 patients with advanced TNBC (median number of lines of therapy in the advanced setting was one, range 0-8), 12% tested positive for the AR. The 6-month clinical benefit rate (CBR) in 26 evaluable, AR positive patients was 19% (95% CI 7-39%) and median PFS 12 weeks (95% CI 11-22 weeks). Subsequent correlative biomarker work by gene expression analysis on 21/26 archival FFPE tissue samples found there was little correlation of AR positivity and LAR positivity as measured by the original TNBC subtype classification with clinical benefit seen in all 6 subtypes with the exception of BL-1 and BL-2. However, it is unclear how the AR positivity would correlate based on the refined TNBC molecular subtype classification where the LAR subtype is identified in a slightly higher proportion of TNBC (12% using the old classification versus 16% using the revised classification).

Enzalutamide, a more potent AR inhibitor, was studied in a phase II trial in patients with advanced AR positive TNBC. Over 50% of patients received enzalutamide as first or second line therapy in the advanced setting. AR expression was identified in 55% of patients. The primary endpoint of CBR at 24 weeks in 75 evaluable patients was 29% (95% CI, 20-41%) and median PFS 14 weeks (95% CI, 8-19).[35] Correlative biomarker work of an androgen-driven gene signature, PREDICT AR, developed via gene expression profiling found that of the 118 patients who received enzalutamide, 56 (47%) were PREDICT AR positive. These patients had a superior 24-week CBR compared to PREDICT AR negative (36% [95% CI, 24-49] versus 7% [95% CI, 2-16]) and better median PFS (16 weeks [95% CI, 10-32] versus 8 weeks [95% CI, 7-13]). The correlation between AR positivity as defined by IHC versus the gene expression signature was not reported. The PREDICT AR gene expression signature has subsequently been shown to have potential prognostic and predictive value in this cohort of TNBC patients.[36]

In a phase II trial of the CYP17A1 inhibitor, abiraterone acetate, plus prednisone in patients with AR positive locally advanced or metastatic TNBC, 53 of 138 (37.6%) patients were AR positive. The 6-month CBR was 20% (95% CI 7.7-38.6%) and median PFS 2.8 months (95% CI, 1.7-5.4%). Median number of lines of therapy in the advanced setting was 2.5 (range 1-9).[37] At the time of analysis, 5 patients remained on treatment with CBR lasting between 6.4 and 24 months.

Approxiamtely 80% of patients with TNBC are basal-like by PAM50 subtype and are associated with high genomic instability, *p53* mutations and poorer prognosis. The remaining

20% are non-basal-like by PAM50, with higher frequency of *PIK3CA* mutations and associated with better prognosis.[38] Pre-clinical research has identified a higher frequency of *PIK3CA* mutations in AR positive TNBC compared to AR negative TNBC (40% versus 4%) and that the combination of anti-androgens and PI3K/mTOR inhibition has additive effects resulting in decreased tumour cell growth.[39] The combination of enzalutamide and the PI3K inhibitor, taselisib, is currently being studied in an early phase trial of patients with advanced AR positive TNBC (NCT02457910).

CDK4/6 inhibitors, involved in cell cycle control, are also generating interest in AR positive TNBC. In pre-clinical studies the LAR subtype was especially sensitive to cell cycle inhibition with the CDK 4/6 inhibitor, palbociclib, and synergy was seen between PI3K and CDK4/6 inhibition in *PIK3CA* mutated TNBC cell lines.[40] A phase I/II trial of palbociclib plus bicalutamide for patients with AR positive, metastatic TNBC is currently recruiting (NCT02605486).

Anti-angiogenic agents and Epidermal growth factor receptor (EGFR) inhibitors

Vascular endothelial growth factor (VEGF) receptor inhibitors

Although initial phase III studies of bevacizumab which targets the vascular endothelial growth factor (VEGF)-mediated angiogenesis pathway showed promise in metastatic breast cancer,[41–43] phase III clinical trials of agents targeting this pathway in TNBC failed to meet their primary endpoints in the adjuvant and metastatic setting.[44,45]

Epidermal growth factor receptor (EGFR) inhibitors

TNBC is associated with overexpression of the epidermal growth factor receptor (EGFR) in approximately 70% of patients[46,47] although *EGFR* gene mutations are rare.[48,49] Phase II clinical trials of the TKIs erlotinib and gefitinib in an unselected metastatic breast cancer population have been reported and showed limited efficacy[50–52] or were closed early due to poor accrual (NCT00739063, NCT01272141). Afatanib, an irreversible TKI, showed minimal activity in a small cohort of heavily pre-treated metastatic TNBC patients[53] however, pre-clinical data and a phase I trial in patients with solid tumours showed synergy when afatinib was combined with paclitaxel.[54] A phase II trial of neoadjuvant afatanib and paclitaxel in TNBC is currently underway (NCT02511847).

Cetuximab and panitumumab, monoclonal antibody inhibitors of EGFR, have had mostly disappointing results in phase II clinical trials in metastatic TNBC with three studies showing limited benefit with respect to ORR and median PFS.[55–57] One randomized phase II trial of cetuximab with or without cisplatin in metastatic

TNBC resulted in a doubling of response rates with ORR (20% versus 10%) with modest improvement in PFS and no improvement in OS.[58]

Although targeting the VEGF and EGFR pathways in TNBC has not shown clinically relevant benefit, ongoing research in understanding the role of these pathways in TNBC may rejuvenate this approach. In the case of targeting EGFR, the role of the immune system may prove to be an integral component and synergy with drug combinations may prove to be effective.[59]

EMERGING TARGETS

Receptor tyrosine kinases - MET

MET is a proto-oncogene of the receptor tyrosine kinase family and becomes overexpressed in a number of tumour types resulting in activation of multiple signal transduction pathways including the MAPK and PI3K pathways that leads to cell growth, proliferation and anti-apoptosis.

Cabozantinib is a multi-targeted TKI against the MET protein amongst others such as RET and VEGFR-2. MET overexpression is associated with basal- like breast cancers in pre-clinical models and associated with worse outcomes.[60] In a phase II study[61] of cabozantinib monotherapy in 35 patients with metastatic TNBC and ≤3 lines of therapy in the advanced setting, the CBR was 31% (95% CI, 17 to 49%). Common toxicities included fatigue, diarrhea, mucositis and palmar-plantar erthrodysesthesia; no grade 4 toxicities were reported. In an update of correlative biomarker work presented at ASCO 2016, cabozantinib was associated with evidence of immune system activation leading to the hypothesis that the combination of cabozantinib with immunotherapy may be beneficial and is hypothesis generating.[62]

The combination of MET and EGFR inhibition in TNBC/basal-like breast cancer has demonstrated activity in 2 pre-clinical studies where cell lines resistant to MET inhibition responded to the addition of erlotinib suggesting synergy and is hypothesis generating.[63,64]

MAPK pathway

The MAPK pathway is one of the most aberrantly expressed pathways in human malignancy resulting in the growth and survival of tumour cells. As part of the MAPK pathway (RAS-RAF-MEK-ERK), MEK kinase is part of a chain of proteins carrying cell surface signals to the nucleus where it results in DNA gene transcription and cell proliferation. This pathway appears to be more active in TNBC/basal-like breast cancer compared to other breast cancer subtypes based on pre-clinical work[65,66] and inhibition of MEK in TNBC cell lines has shown activity.[65,67] Taxane resistance is common in TNBC and preclinical data suggest this may be due, in part, to up-regulation of the

MAPK pathway.[68] The addition of a MEK inhibitor may reverse resistance. The phase II COLET trial[69] is currently assessing the role of the MEK inhibitor, cobimetinib plus paclitaxel in the first line treatment of locally advanced/metastatic TNBC. Fourteen patients were evaluable in the first stage of this clinical trial; 8 patients had a partial response, with stable disease and progressive disease in 3 patients each. The most common toxicities were diarrhea (63%), rash (50%), nausea (44%), alopecia (31%), pyrexia, asthenia, dyspnea, peripheral edema, stomatitis, vomiting and constipation (all 25%). Grade 3 adverse events (AE) occurred in 9 (56%) patients. No grade 4/5 AEs were reported. A phase II trial of selumetinib plus docetaxel in addition to AC chemotherapy in the neoadjuvant setting for patients with TNBC is being planned and will assess pCR rates (NCT02685657).

MEK inhibitors have shown synergy in pre-clinical models when administered in combination with other targeted therapies such EGFR and AKT inhibitors.[70] A phase II clinical trial is ongoing in metastatic breast cancer with the MEK inhibitor trametinib plus the AKT inhibitor, GSK2141795 (NCT01964924).

JAK/STAT pathway

Targeting the JAK/STAT pathway is also garnering interest in TNBC. Through next generation sequencing Balko et al.[71] have identified higher rates of amplifications or gains at the 9p24.1 locus, which includes the Janus kinase 2 (JAK2), in patients with residual disease after neoadjuvant chemotherapy compared to untreated TNBC suggesting a causal association with chemotherapy resistance. In cell lines treated with the general JAK inhibitor, ruxolitinib, tumour growth was not halted but BSK805, a JAK-2 specific inhibitor, resulted in decreased TNBC tumour growth when paired with chemotherapy. Early phase studies of neoadjuvant ruxolitinib plus paclitaxel or paclitaxel/AC in inflammatory TNBC are ongoing or pending accrual (NCT02041429, NCT02876302). More JAK-2 specific inhibitors may be required for more efficacious results.

Other pre-clinical work targeting JAK-2 by Barrett et al.[72] has identified a region of overlap in the 9p24.1 locus that includes loci for PD-L1, PD-l2 and JAK-2, referred to as the PDJ amplicon. This amplicon was identified in 12/41 TNBC but absent in ER+ (0/8) and HER2+ (0/15) breast tumours. Therefore, there may be a role for combining JAK-2 inhibition with immune checkpoint therapy with the PDJ amplicon a potential biomarker.

PI3K pathway

AKT is a protein in the PI3K-mTOR-AKT pathway that is aberrantly activated in a subset of patients with TNBC. As part of the adaptive randomization I-SPY 2 trial, the AKT inhibitor, MK-2206, in combination with standard AC and weekly

paclitaxel chemotherapy as neoadjuvant treatment in the TNBC cohort resulted in pCR rates of 40% versus 22% in the standard neoadjuvant chemotherapy arm and a predictive probability of success in phase III trials of 76%.[73] A number of other phase II trials of the AKT inhibitors ipatasertib and AZD5363 in combination with paclitaxel are underway in metastatic TNBC (NCT02162719, NCT02423603) as well as the neoadjuvant setting (NCT02301988).

Antibody drug conjugates (ADC)

Antibody drug conjugates (ADCs) are composed of an antibody attached to active cytotoxic chemotherapy via a linker molecule and allows for cytotoxic chemotherapy to be directed to cancer cells bearing a specific marker thus sparing normal tissue from excessive toxicity.

The phase I/II trial results of the ADC, sacituzumab govitecan (IMMU-132), in patients with heavily pre-treated metastatic TNBC was presented at ASCO 2015.[74] This ADC is a combination of the humanized anti-Trop-2 monoclonal antibody coupled to SN-38, the active metabolite of irinotecan, which targets cells with high expression of Trop-2 which includes a high proportion of patients with TNBC. In the cohort of 48 patients with TNBC, the ORR was 21% and CBR (CR+PR+SD ≥6 months) 37%. Grade 3/4 toxicity included neutropenia (30%), febrile neutropenia (3%), diarrhea, anemia, leucopenia, lymphopenia, caecitis (all 3%).

Glembatumumab vedotin (GV), another ADC containing monomethylauristatin E (MMAE) as the active chemotherapeutic agent, showed promise in a phase II clinical trial[75] of patients with heavily pre-treated metastatic breast cancer. In an unplanned analysis, ORR was 40% (GV arm) versus 0% (investigator's choice arm) for patients with glycoprotein NMB (gpNMB) overexpressing TNBC. This internalizable transmembrane protein is overexpressed in up to 40% of patients with TNBC and associated with poorer prognosis. GV was associated with less hematologic toxicity than IMMU-132; common toxicities included rash, pruritis, neuropathy and alopecia. GV is currently being investigated in a randomized phase II trial (METRIC) for patients with metastatic gpNMB-overexpressing TNBC versus capecitabine as the comparator arm randomized in a 2:1 fashion. The primary endpoint is PFS; secondary endpoints include ORR, OS, duration ofresponse, and pharmacokinetics/ pharmacodynamics (NCT01997333).

Wnt, NOTCH and STAT3 pathways, glucocorticoid receptor

The Wnt signaling pathway (NCT01973309), STAT3 pathway (NCT01325441), NOTCH pathway (NCT02299635) and glucocorticoid receptor (NCT02014337) represent

other potential targets in TNBC with early phase clinical trials underway.

Discussion

TNBC is an extremely heterogeneous disease that has made the development of targeted therapies an exceptionally difficult challenge. While patients with hormone sensitive or HER2 amplified breast cancer have a number of targeted therapy options, TNBC has yet to see a single approved targeted agent and cytotoxic chemotherapy remains the standard of care. However, a number of targeted therapies for the treatment of TNBC are showing promise, either as monotherapy, or in combination with other targeted therapy or chemotherapy. The treatment advancements outlined in this review are in large part due to ongoing research via gene expression analysis and other large scale genomic projects that have begun to unravel the complex underlying biology of TNBC.

Although it has been known for some time that DNA damaging therapies such as platinum salts and PARP inhibitors are active in patients with germline *BRCA* mutations, assays are in development to predict *BRCA* WT patients who, due to deficiencies in HR, may benefit from these agents. A biomarker of HR deficiency will likely be available for clinical use in the foreseeable future. As we come to understand which patients harbor "BRCA-like" tumours, the next step is the development of novel therapies that target the HR pathway. Research in this area is underway including a Stand Up To Cancer Canada collaborative effort actively developing 3 novel drugs targeting this tumour vulnerability.

TNBC is considered an aggressive form of breast cancer that often responds well initially to cytotoxic chemotherapy. While the majority (80%) of TNBC are basal-like by PAM50, associated with high levels of genomic instability, *p53* mutations, and poor prognosis, the remaining 20% are non-basal like by PAM50, associated with lower levels of genomic instability, *PIK3CA* mutations, less response to cytotoxic chemotherapy, and a better prognosis. There is evidence that patients who are AR positive have a higher proportion of *PIK3CA* mutations and may derive benefit from anti-androgen based therapy. The LAR subtype by gene expression analysis appears to overlap to some extent with AR positivity (by IHC) but the best method by which to determine AR positivity is unknown and is an active area of research.

TNBC is often associated with high levels of genomic instability and TILs, both of which are associated with sensitivity to immune checkpoint blockade. Early phase clinical trials of immune checkpoint blockade as monotherapy have yielded modest ORR (< 20%) albeit in patients who were heavily pre-treated. Based on results of improved ORR in early phase clinical trials, attention is now

focused on combining immune checkpoint blockade with cytotoxic chemotherapy, immune modulators and vaccines as well as dual immune checkpoint inhibition. A predictive biomarker has not been clearly identified and research in this area is ongoing.

Many of the emerging targets (eg. MAPK, PI3K and JAK/STAT pathways) are in very early phase clinical trials. Although targeting these pathways has biologic rationale, the results of clinical trials will determine whether these drugs will be efficacious in select patients with TNBC and satisfy regulatory requirments .

Conclusions

Ongoing research into the underlying biology of TNBC is needed to continue the current trajectory of emerging and promising targeted therapies for this heterogeneous disease. Correlative biomarker research will aid in selecting patients who are likely to benefit from novel (and not so novel) drug therapies.

REFERENCES

1. Gonzalez-Angulo AM, Timms KM, Liu S, et al. Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. *Clin Cancer Res*. 2011;17(5):1082-1089. doi:10.1158/1078-0432.CCR-10-2560.

2. Krejci L, Altmannova V, Spirek M, Zhao X. Homologous recombination and its regulation. *Nucleic Acids Res*. 2012;40(13):5795-5818. doi:10.1093/nar/gks270.

3. Akashi-Tanaka S, Watanabe C, Takamaru T et al. BRCAness predicts resistance to taxane-containing regimens in triple negative breast cancer during neoadjuvant chemotherapy. *Clin Breast Cancer*. 2015;15(1):80-85.

4. Lehmann BDB, Bauer J a J, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest*. 2011;121(7):2750-2767. doi:10.1172/JCI45014DS1.

5. Lehmann BD, Jovanović B, Chen X, et al. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. *PLoS One*. 2016;11(6):e0157368. doi:10.1371/journal.pone.0157368.

6. Von Minckwitz G, Schneeweiss A, Loibl S, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): A randomised phase 2 trial. *Lancet Oncol*. 2014;15(7):747-756. doi:10.1016/S1470-2045(14)70160-3.

7. Kaklamani VG, Jeruss JS, Hughes E, et al. Phase II neoadjuvant clinical trial of carboplatin and eribulin in women with triple negative early-stage breast cancer (NCT01372579). *Breast Cancer Res Treat*. 2015;151(3):629-638. doi:10.1007/s10549-015-3435-y.

8. von Minckwitz G, Oibl S, Schneesweiss A, et al. Early survival analysis of the randomized phase II trial investigating the addition of carboplatin to neoadjuvant therapy for triple-negative and HER2-positive early breast cancer (GeparSixto). *Proceedings of the Thirty-Eighth Annual CTRC-AACR San Antonio Breast Cancer Symposium: 2015 Dec 8-12; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res* 2016;76(4 Suppl):Abstract nr S2-04.

9. Sikov WM, Berry DA, Perou CM, et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (A. *J Clin Oncol*. 2015;33(1):13-21. doi:10.1200/JCO.2014.57.0572.

10. Telli ML, McMillan A, Ford JM et al. [P3-07-12] Homologous recombination deficiency (HRD) as a predictive biomarker of response to

neoadjuvant platinum-based therapy in patients with triple negative breast cancer (TNBC): A pooled analysis. *Proceedings of the Thirty-Eighth Annual CTRC-AACR San Antonio Breast Cancer Symposium: 2015 Dec 8-12; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res* 2016;76(4 Suppl):Abstract nr P3-07-12.

11. Connolly R, Elkin E, Timms K et al. Homologous recombination deficiency (HRD) as a predictive biomarker of response to preoperative systemic therapy (PST) in TBCRC008 comprising a platinum in HER2-negative primary operable breast cancer. *Proceedings of the Thirty-Eighth Annual CTRC-AACR San Antonio Breast Cancer Symposium: 2015 Dec 8-12; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res* 2016;76(4 Suppl):Abstract nr P3-07-13.

12. Tutt A, Ellis P, Kilburn L et al. Abstract S3-01: The TNT trial: A randomized phase III trial of carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or BRCA1/2 breast cancer (CRUK/07/012). *Cancer Res*. 2015;75:S3-01. doi:10.1158/1538-7445.SABCS14-S3-01.

13. O'Shhaughnessy J, Schwartzberg L, Danso M, et al. Phase III study of iniparib plus gemcitabine and carboplatin versus gemcitabine and carboplatin in patients with metastatic triple-negative breast cancer. *J Clin Oncol.* 2014;32(34):3840-3847.

14. Tutt A, Robson M, Garber JE, et al. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. *Lancet*. 2010;376(9737):235-244. doi:10.1016/S0140-6736(10)60892-6.

15. Gelmon K a., Tischkowitz M, Mackay H, et al. Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: A phase 2, multicentre, open-label, non-randomised study. *Lancet Oncol*. 2011;12(9):852-861. doi:10.1016/S1470-2045(11)70214-5.

16. Robson M, Tutt A, Balmana J et al. OlympiA, Neo-Olympia and OlympiAD: Randomized phase III trials of olaparib in patients (pts) with breast cancer (BC) and a germline BRCA1/2 mutation (gBRCAm). *Proceedings of the Thirty-Seventh Annual CTRC-AACR San Antonio Breast Cancer Symposium: 2014 Dec 9-13; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res* 2015;75(9 Suppl):Abstract nr OT1-1-04.

17. Rugo HS, Olopade OI, DeMichele A, et al. Adaptive Randomization of Veliparib–Carboplatin Treatment in Breast Cancer. *N Engl J Med*. 2016;375(1):23-34. doi:10.1056/NEJMoa1513749.

18. van t' Veer L, Esserman L, Sanil A, et al. DNA repair deficiency biomarkers identify HR + / HER2- breast cancer patients who may benefit from veliparib / carboplatin :

results from the I-SPY 2 TRIAL. *J Clin Oncol*. 2015;33:(suppl; abstr 521).
19. Miller K, Tong Y, Jones DR et al. Cisplatin with or without rucaparib after preoperative chemotherapy in patients with triple negative breast cancer: Final efficacy results if Hoosier Oncology Group BRE09-146. *J Clin Oncol*. 2015;33(suppl; abstr 1082).
20. Rizvi NA, Hellmann MD, Snyder A, et al. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015; 348(6230):124-128. doi:10.1126/science.aaa1348.
21. Snyder A, Makarov V, Merghoub T et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 371(23):2189-2199. doi:10.1056/NEJMoa1406498.
22. Ibrahim EM, Al-Foheidi ME, Al-Mansour MM, Kazkaz GA. The prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancer: a meta-analysis. *Breast Cancer Res Treat*. 2014;148(3):467-476. doi:10.1007/s10549-014-3185-2.
23. Nanda R, Chow LQM, Dees EC, et al. Pembrolizumab in Patients With Advanced Triple-Negative Breast Cancer: Phase Ib KEYNOTE-012 Study. *J Clin Oncol*. 2016;34(21):1-10. doi:10.1200/JCO.2015.64.8931.
24. Emens LA, Braiteh FS, Cassier P et al. Inhibition of PD-L1 by MPDL3280A leads to clinical activity in patients with metastatic triple-negative breast cancer (TNBC). *Proceedings of the 106th Annual Meeting of the American Association for Cancer Research; 2015 Apr 18-22; Philadelphia, PA. Philadelphia (PA): AACR; Cancer Res* 2015;75(15 Suppl):Abstract nr 2859. doi:10.1158/1538-7445.AM2015-2859
25. Chen DS, Mellman I. Oncology meets immunology: The cancer-immunity cycle. *Immunity*. 2013;39(1):1-10. doi:10.1016/j.immuni.2013.07.012.
26. Hodge JW, Garnett CT, Farsaci B, et al. Chemotherapy-induced immunogenic modulation of tumor cells enhances killing by cytotoxic T lymphocytes and is distinct from immunogenic cell death. *Int J Cancer*. 2013;133(3):624-636. doi:10.1002/ijc.28070.
27. Adams S, Diamond J, Hamilton EP et al. Phase Ib trial of atezolizumab in combination with nab-paclitaxel in patients with metastatic triple-negative breast cancer (mTNBC). *J Clin Oncol*. 2016;34:(suppl; abstr 1009).
28. Vera-Badillo FE, Templeton AJ, De Gouveia P, et al. Androgen receptor expression and outcomes in early breast cancer: A systematic review and meta-analysis. *J Natl Cancer Inst*. 2014;106(1):1-11. doi:10.1093/jnci/djt319.
29. Hickey TE, Robinson JLL, Carroll JS, Tilley WD. Minireview: The androgen receptor in breast tissues: growth inhibitor, tumor suppressor, oncogene? *Mol Endocrinol*. 2012;26(8):1252-1267. doi:10.1210/me.2012-1107.
30. Rampurwala M, Wisinski KB, O'Regan R. Role of the androgen receptor in triple-negative breast cancer. *Clin Adv Hematol Oncol*.

2016;14(3):186-193.
31. ézéquel P, Loussouarn D, Guérin-Charbonnel C, et al. Gene-expression molecular subtyping of triple-negative breast cancer tumours: importance of immune response. *Breast Cancer Res*. 2015;17(2015):43. doi:10.1186/s13058-015-0550-y.
32. Barton VN, D'Amato NC, Gordon MA, et al. Multiple Molecular Subtypes of Triple-Negative Breast Cancer Critically Rely on Androgen Receptor and Respond to Enzalutamide In Vivo. *Mol Cancer Ther*. 2015;14(3):[Epub ahead of print]. doi:10.1158/1535-7163.MCT-14-0926.
33. Gilmore H, Varadan V, Williams N et al. Androgen receptor expression in triple negative breast cancer. *Proceedings of the Thirty-Seventh Annual CTRC-AACR San Antonio Breast Cancer Symposium: 2014 Dec 9-13; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res* 2015;75(9 Suppl):Abstract nr P3-04-07.
34. Gucalp A, Tolaney S, Isakoff SJ, et al. Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. *Clin Cancer Res*. 2013;19(19):5505-5512. doi:10.1158/1078-0432.CCR-12-3327.
35. Traina TA, Miller K, Yardley DA et al. Results from a phase 2 study of enzalutamide (ENZA), an androgen receptor (AR) inhibitor, in advanced AR+ triple-negative breast cancer (TNBC). *J Clin Oncol*. 33(suppl; abstr 1003).
36. Miller K, Krop I, Schwartzberg L et al. Improved clinical outcomes on enzalutamide observed in patients with PREDICT AR+ triple-negative breast cancer: prognosis or prediction? *Proceedings of the Thirty-Eighth Annual CTRC-AACR San Antonio Breast Cancer Symposium: 2015 Dec 8-12; San Antonio, TX. Philadelphia (PA): AACR; Cancer Res* 2016;76(4 Suppl):Abstract nr P3-07-25.
37. Bonnefoi H, Grellety T, Tredan O, et al. A phase II trial of abiraterone acetate plus prednisone in patients with triple-negative androgen receptor positive locally advanced or metastatic breast cancer (UCBG 12-1). *Ann Oncol*. 2016;27(5):812-818. doi:10.1093/annonc/mdw067.
38. Xu H, Eirew P, Mullaly SC, Aparicio S. The omics of triple-negative breast cancers. *Clin Chem*. 2014;60(1):122-133. doi:10.1373/clinchem.2013.207167.
39. Lehmann BD, Bauer JA, Schafer JM, et al. PIK3CA mutations in androgen receptor-positive triple negative breast cancer confer sensitivity to the combination of PI3K and androgen receptor inhibitors. *Breast Cancer Res*. 2014;16(4):406. doi:10.1186/s13058-014-0406-x.
40. Asghar U, Herrera-Abreu T, Cutts R et al. Identification of subtypes of triple negative breast cancer (TNBC) that are sensitive to CDK4/6 inhibition. *J Clin Oncol*. 2015;33(suppl; abstr 11098).
41. Miller K, Wang M, Gralow J et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med*. 2007;357:2666-2676.
42. Miles DW, Chan A, Dirix LY et

al. Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. *J Clin Oncol*. 2010;28:3239-3247.

43. Robert NJ, Dieras V, Glaspy J et al. RIBBON-1: Randomized, double-blind, placebo-controlled, phase III trial of chemotherapy with or without bevacizumab for first-line treatment of human epidermal growth factor 2-negative, locally recurrent or metastatic breast cancer. *J Clin Oncol*. 2011;29:1252-1260.

44. Cameron D, Brown J, Dent R, et al. Adjuvant bevacizumab-containing therapy in triple-negative breast cancer (BEATRICE): Primary results of a randomised, phase 3 trial. *Lancet Oncol*. 2013;14(10):933-942. doi:10.1016/S1470-2045(13)70335-8.

45. Mackey JR, Ramos-Vazquez M, Lipatov O, et al. Primary results of ROSE/TRIO-12, a randomized placebo-controlled phase III trial evaluating the addition of ramucirumab to first-line docetaxel chemotherapy in metastatic breast cancer. *J Clin Oncol*. 2015;33(2):141-148. doi:10.1200/JCO.2014.57.1513.

46. Choi J, Jung WH, Koo JS et al. Clinicopathologic features of molecular subtypes of triple negative breast cancer based on immunohistochemical markers. *Histol Histopathol*. 2012;27:1481-1493.

47. Gumuskaya B, Alper M, Hucumenoglu S et al. EGFR expression and gene copy number in triple-negative breast carcinoma. *Cancer Genet Cytogenet*. 2010;203:222-229.

48. Grob TJ, Heilenkotter U, Geist S, et al. Rare oncogenic mutations of predictive markers for targeted therapy in triple-negative breast cancer. *Breast Cancer Res Treat*. 2012;134:561-567.

49. Jacot W, Lopez-Crapez E, Thezenas S, et al. Lack of EGFR-activating mutations in European patients with triple-negative breast cancer could emphasise geographic and ethnic variations in breast cancer mutation profiles. *Breast Cancer Res*. 2011;13:R133.

50. von Minckwitz G, Jonat W, Fasching P, et al. A multicentre phase II study gefitinib in taxane- and anthracycline-pretreated metastatic breast cancer. *Breast Cancer Res Treat*. 89(2):165-172.

51. Baselga J, Albanell J, Ruiz A, et al. Phase II and tumor pharmacodynamic study of gefitinib in patients with advanced breast cancer. *J Clin Oncol*. 2005;23(23):5323-5333. doi:10.1200/JCO.2005.08.326.

52. Dickler MN, Cobleigh MA, Miller KD, Klein PM, Winer EP. Efficacy and safety of erlotinib in patients with locally advanced or metastatic breast cancer. *Breast Cancer Res Treat.* 2009;115(1):115-121. doi:10.1007/s10549-008-0055-953.

53. Schuler M, Awada A, Harter P, et al. A phase II trial to assess efficacy and safety of afatinib in extensively pretreated patients with HER2-negative metastatic breast cancer.

Breast Cancer Res Treat. 2012;134(3):1149-1159. doi:10.1007/s10549-012-2126-1.

54. Suder A, Ang JE, Kyle F, et al. A phase I study of daily afatinib, an irreversible ErbB family blocker, in combination with weekly paclitaxel in patients with advanced solid tumours. *Eur J Cancer*. 51(16):2275-2284. doi:10.1016/j.ejca.2015.07.041.

55. Carey LA, Rugo HS, Marcom PK, et al. TBCRC 001: Randomized phase II study of cetuximab in combination with carboplatin in stage IV triple-negative breast cancer. *J Clin Oncol*. 2012;30(21):2615-2623. doi:10.1200/JCO.2010.34.5579.

56. Tredan O, Campone M, Jassem J, et al. Ixabepilone alone or with cetuximab as first-line treatment for advanced/metastatic triple-negative breast cancer. *Clin Breast Cancer*. 2015;15(1):8-15. doi:10.1016/j.clbc.2014.07.007.

57. Yardley DA, Ward PJ, Daniel BR, et al. Panitumumab, Gemcitabine, and Carboplatin as Treatment for Women With Metastatic Triple-Negative Breast Cancer: A Sarah Cannon Research Institute Phase II Trial. *Clin Breast Cancer*. 2016:1-7. doi:10.1016/j.clbc.2016.05.006.

58. Baselga J, Gomez P, Greil R, et al. Randomized phase II study of the anti-epidermal growth factor receptor monoclonal antibody cetuximab with cisplatin versus cisplatin alone in patients with metastatic triple-negative breast cancer. *J Clin Oncol*. 2013;31(20):2586-2592. doi:10.1200/JCO.2012.46.2408.

59. Nabholtz JM, Chalabi N, Radosevic-Robin N et al. Multicentric neoadjuvant pilot Phase II study of cetuximab combined with docetaxel in operable triple negative breast cancer. *Int J Cancer*. 138(9):2274-2280.

60. Graveel CR, DeGroot JD, Su Y et al. Met induces mammary tumors with diverse histologies and is associated with poor outcome and human basal breast cancer. *Proc Natl Acad Sci U S A*. 2009;106(31):12903-12908. doi:10.1073/pnas.0810402106.

61. Tolaney SAM, Ziehr DR, Gou H et al. A phase II study of cabozantinib for metastatic triple-negative breast cancer (TNBC). *J Clin Oncol*. 2015;33(suppl; abstr 1080).

62. Duda DG, Ziehr DR, Guo H et al. Effect of cabozantinib treatment on circulating immune cell populations in patients with metastatic triple-negative breast cancer (TNBC). *J clin Oncol*. 2016;34:(suppl; abstr 1093).

63. Yi YW, You K, Bae EJ, Kwak S-J, Seong Y-S, Bae I. Dual inhibition of EGFR and MET induces synthetic lethality in triple-negative breast cancer cells through downregulation of ribosomal protein S6. *Int J Oncol*. 2015:1-11. doi:10.3892/ijo.2015.2982.

64. Kim YJ, Choi JS, Seo J, et al. MET is a potential target for use in combination therapy with EGFR inhibition in triple-negative/basal-like breast cancer. *Int J Cancer*. 2014;134(10):2424-2436. doi:10.1002/ijc.28566.

65. Balko JM, Cook RS, Vaught DB et al. Profiling of residual breast cancers after neoadjuvant chemotherapy identifies DUSP4

deficiency as a mechanism of drug resistance. *Nat Med*. 2012;18(7):1052-1059.

66. Hoeflich KP, O'brien C, Boyd Z et al. In vivo antitumor activity of MEK and phosphatidylinositol 3-kinase inhibitors in basal-like breast cancer models. *Clin Cancer Res*. 2009;15(14):4649-4664.

67. Jing J, Greshock J, Holbrook JD et al. Comprehensive predictive biomarker analysis for MEK inhibitor GSK1120212. *Mol Cancer Ther*. 2012;11(3):720-729.

68. Menendez J, Vellon L, Mehmi I, Teng PK, Griggs DW, Lupu R. A novel CYR61-triggered 'CYR61-alphavbeta3 integrin loop' regulates breast cancer cell survival and chemosensitivity through activation of ERK1/ERK2 MAPK signaling pathway. *Oncogene*. 2005;24(5):761-779. doi:10.1038/sj.onc.1208238.

69. Brufsky A, Kim S, Thierry J et al. Cobimetinib (C) + paclitaxel (P) as first-line treatment in patients (pts) with advanced triple-negative breast cancer (TNBC): Updated results and biomarker data from the phase 2 COLET study. *J Clin Oncol*. 2016;34:(suppl; abstr 1074).

70. Maiello MR, Amelia D, Bevilacqua S, Gallo M, Normanno N, De Luca A. EGFR and MEK Blockade in Triple Negative Breast Cancer Cells. *J Cell Biochem*. 2015;2785(April):1-25. doi:10.1002/jcb.25220.

71. Balko JM, Schwarz LJ, Luo N, et al. Triple-negative breast cancers with amplification of JAK2 at the 9p24 locus demonstrate JAK2-specific dependence. *Sci Transl Med*. 2016;8(334):334ra53. doi:10.1126/scitranslmed.aad3001.

72. Barrett MT, Anderson KS, Lenkiewicz E, et al. Genomic amplification of 9p24.1 targeting JAK2, PD-L1, and PD-L2 is enriched in high-risk triple negative breast cancer. *Oncotarget.* 2015;6(28):1-11.

73. Tripathy D, Chien AJ, Hylton N et al. Adaptively randomized trial of neoadjuvant chemotherapy with or without the Akt inhibitor MK-2206: Graduation results fromt the I-SPY 2 Trial. *J Clin Oncol*. 2015;33(suppl; abstr 524).

74. Bardia A, Vahdat LT, Diamond JR et al. Therapy of refractory/relapsed metastatic triple-negative breast cancer (TNBC) with an anti-Trop-2-SN-38 antibody-drug conjugate (ADC), sacituzumab govitecan (IMMU-132): Phase I/II clinical experience. *J Clin Oncol*. 2015;33:(suppl; abstr 1016).

75. Yardley DA, Weaver R, Melisko ME, et al. EMERGE: A randomized phase II study of the antibody-drug conjugate glembatumumab vedotin in advanced glycoprotein NMB - Expressing breast cancer. *J Clin Oncol*. 2015;33(14):1609-1619. doi:10.1200/JCO.2014.56.2959.

