Precision Breast Cancer Medicine: early stage triple negative breast cancer -a review of molecular characterisation, therapeutic targets and future trends

Karen Pinilla Alba[1, 2, 3], Lynsey M. Drewett[1, 2], Rebecca Lucey[1, 2], Jean E. Abraham[1, 2, 3*]

[1]Cambridge Breast Cancer Research Unit, University of Cambridge, Department of Oncology, United Kingdom, [2]Cambridge University Hospitals NHS Foundation Trust, United Kingdom, [3]Cancer Research UK Cambridge Centre, Cancer Research UK Cambridge Institute, University of Cambridge, United Kingdom

Submitted to Journal:
Frontiers in Oncology

Specialty Section:
Breast Cancer

Article type:
Review Article

Manuscript ID:
866889

Received on:
31 Jan 2022

Revised on:
18 Apr 2022

Journal website link:
www.frontiersin.org

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

KP, LD, RL, and JA each contributed to the design, literature review, writing, and editing of the manuscript. All authors agree to be accountable for the content of the work.

Keywords

Breast, Cancer, Precision, triple, negative, therapeutic, target, biomarkers

Abstract

Word count: 301

Personalised approaches to the management of all solid tumours are increasing rapidly along with wider accessibility for clinicians. Advances in tumour characterisation and targeted therapies have placed triple negative breast cancers (TNBC) at the forefront of this approach. TNBC is a highly heterogeneous disease with a variety of histopathological features and is driven by distinct molecular alterations. The ability to tailor individualised and effective treatments for each patient based is of particular importance in this group due to the high risk of distant recurrence and death.

The mainstay of treatment across all subtypes of TNBC has historically been cytotoxic chemotherapy which is often associated with off-target tissue toxicity and drug resistance. Neoadjuvant chemotherapy is commonly used as it allows close monitoring of early treatment response and provides valuable prognostic information. Patients who achieve a complete pathological response after neoadjuvant chemotherapy are known to have significantly improved long-term outcomes. Conversely, poor responders are known to face a higher risk of relapse and death. The identification of those subgroups that are more likely to benefit from breakthroughs in the personalised approach is one of the challenges of the current era where several targeted therapies are available.

The aim of this review is to present an overview of contemporary practice, and promising future trends in the management of early TNBC. Platinum chemotherapy, DNA damage response (DDR) inhibitors, immune checkpoint inhibitors, inhibitors of the PI3K-AKT-mTOR, and Androgen receptor (AR) pathways are some of the increasingly studied therapies which will be reviewed. We will also discuss the growing evidence for less-developed agents and predictive biomarkers that are likely to contribute to the forthcoming advances in this field. Finally, we will propose a framework for the personalised management of TNBC based upon the integration of clinico-pathological and molecular features to ensure that long term outcomes are optimised.

Contribution to the field

Triple negative breast cancer (TNBC) accounts for 15% of all breast cancers. It affects younger patients, has an aggressive natural history, and presents at an advanced stage. It is a highly heterogeneous disease and exhibits a wide variety of features that can be characterised based upon genetics and pathology, at both the tissue and the molecular level. These features have evolved over time and with increasing understanding of the biological landscape of the disease, although they are not exploited in the clinical setting. This review describes the development of this multifaceted categorisation and draws upon it to focus on current and promising future treatment approaches. The evidence base for well-established chemotherapy regimens is reviewed. Newer treatments and clinical trial progress to date is summarised. We argue that the current therapeutic options for TNBC are limited. Patients diagnosed with early stage disease will far too frequently have limited response to routinely prescribed treatment and face poor long-term outcomes. We highlight the importance of the personalised approach to managing the disease and propose a framework to guide the clinician towards developing a bespoke treatment pathway for every patient diagnosed with early stage TNBC.

Funding statement

We would like to thank and acknowledge our funders Cancer Research UK (CRUK) Cambridge Centre Grant Funding: RDZD141, RDZD192); Addenbrookes Charitable Trust (98100), and the Mark Foundation for Cancer Research (RDZD/174).
This research was also supported by the NIHR Cambridge Biomedical Research Centre (BRC-1215-20014). The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

Precision Breast Cancer Medicine: early stage triple negative breast cancer - a review of molecular characterisation, therapeutic targets and future trends

Karen Pinilla Alba[1,2,3], Lynsey M Drewett[1,2], Rebecca Lucey[1,2], Jean E Abraham[1,2,3]

[1] Precision Breast Cancer Institute, University of Cambridge, Department of Oncology, Cambridge, UK, [2] Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK; [3] Cancer Research UK Cambridge Centre, Cancer Research UK Cambridge Institute, University of Cambridge, Cambridge, UK.

Correspondence:

Professor Jean Abraham
ja344@medschl.cam.ac.uk

Keywords: Breast, cancer, precision, triple, negative, biomarkers, therapeutic, target

1. Introduction

Breast cancer (BC) is the most common cancer affecting women and is the leading cause of cancer-related death in women worldwide[1]. Triple negative breast cancers (TNBC), a highly heterogeneous subtype, represent approximately 15% of all breast cancers[2]. TNBC behaves aggressively, has a poorer prognosis, and a higher risk of distant relapse and death relative to other BC subtypes[2]. Genomic and transcriptomic data has enhanced our ability to understand the TNBC taxonomy and enabled the identification of new therapeutic targets. The development of new therapeutic options and optimisation of personalised management strategies is critical in improving outcomes for affected patients.

This review aims to provide an overview of contemporary practice in the treatment of early-stage TNBC and to highlight promising future directions. The growing evidence for newer therapies predicted to contribute to forthcoming advances in this field will be discussed. Finally, a framework for the personalised management of TNBC based upon the integration of clinical and molecular features will be discussed.

2. Diagnosis and clinical presentation

TNBC is characterised by the absence of oestrogen (ER) and progesterone (PR) receptor expression, in addition to the absence of HER2 amplification as measured by immunohistochemistry or fluorescence in situ hybridisation. TNBC is disproportionately seen in younger women, as well as Hispanic and African American populations[3]. Disease-free intervals following primary treatment of early-stage (I-III) TNBC are often short. The recurrence rate for is 25%, with the highest risk of recurrence in the first three years after diagnosis, and a median time to relapse after surgery of 18.8 months[4]. Metastatic TNBC (mTNBC) exhibits a more aggressive phenotype than other BC subtypes, as demonstrated by a shorter chemotherapy response duration, and a shorter overall survival (OS) (median 13.3 months)[5].

3. TNBC heterogeneity

TNBC is a heterogeneous disease with significant inter- and intra-tumour heterogeneity[6,7,8]. Multiple efforts have focused on adequately addressing this biological complexity to enable the tailoring of therapeutic options to individual tumour characteristics.

3.1. Histological subtypes

The current clinical definition of TNBC encompasses multiple histological subtypes. Approximately 85% of TNBC are morphologically defined as invasive carcinoma of no special type (IC-NST). The remaining TNBCs are less common tumours of special type which are collectively associated with a worse prognosis[9]. Individual special types display distinct pathological and molecular characteristics and prognoses. Tumours of indolent course include adenoid cystic, secretory and tubular carcinomas. Medullary histology is associated with a good prognosis and high response rates to chemotherapy, whereas metaplastic tumours show differentiation toward squamous epithelium with mesenchymal components and are frequently chemoresistant[10]. An accurate histological examination marks the first step towards the identification of key mechanistic features that could be exploited to direct treatment (Table 1).

Table 1: Histological special subtypes of TNBC

3.2. Molecular subtypes

Numerous efforts to build upon the molecular classification of TNBC have been proposed (Table 2). Here we review the most recognised classifiers that utilise genomic and transcriptomic data and summarise their predictive value when tested in early TNBC clinical cohorts. Many other classification approaches have been proposed (Table S1), with the absence of clinical evidence for treatment response limiting their use.

3.2.1. Intrinsic subtypes

Breast cancers can be classified into six intrinsic molecular subtypes by gene expression (GE) profiling[17,18] as follows: Luminal A, Luminal B, Her2 enriched, Normal-like, Basal-like, and Claudin low. Each subtype is identified within the TNBC group as defined by immunohistochemistry. Basal-like tumours are most frequent (50%-75%), however, they are not exclusive to the TNBC phenotype[18]. The claudin-low subtype represents 25-40% of TNBC and was more recently introduced[19].

Basal-like tumours are characterised by the presence of cytokeratins typically expressed by the basal layer of the skin, widespread genomic instability, high proliferation markers, loss of function of *BRCA1*, and dysregulation of *MYC* and *RB1* pathways[18]. Claudin-low tumours have several features in common with basal-like tumours but are uniquely characterised by low levels of cell adhesion proteins, enrichment of mesenchymal traits and stem cell features[20]. Luminal tumours overexpress a 'luminal signature' containing *ESR1, GATA3, FOXA1, XBP1* and *MYB*. Her2 amplification concomitantly with overexpression of *HER2*-amplicon-associated genes defines the Her2 enriched subtype[32].

Intrinsic subtypes provide independent predictive information regarding response to neoadjuvant chemotherapy (NACT) when considering all subtypes of breast cancer, although not consistently for the TNBC cohort when viewed in isolation. Claudin-low tumours are associated with lower pathological complete response (pCR) rates in comparison to basal-like subtypes[21]. In a subgroup analysis of the BrighTNess trial, pCR rates were higher for basal-like vs. non-basal tumours (52.3% vs 35.4%, p=0.003)[21]. In contrast, no difference in pCR rate was observed with the addition of carboplatin for patients with basal-like TNBC vs non-basal TNBC in the CALGB40603 study[22]. These results illustrate that the predictive value often linked to the basal-like subtypes has not always been reproduced in the early setting of TNBC, making intrinsic subtypes a less reliable biomarker of response within this group.

3.2.2. Lehmann/Pietenpol subtypes

Lehmann et al. selected clustering analyses to identify six TNBC subtypes displaying unique GE patterns and ontologies. Each subtype was characterised by the activation of specific signalling pathways that led to a selective response to targeted therapies in vivo[23]. Additional histopathological quantification and laser-capture microdissection prompted a refined classification with only four tumour-specific subtypes (TNBCtype-4). The original immunomodulatory and mesenchymal stem-like subtypes were deemed to originate from infiltrating lymphocytes and tumour-associated stromal cells; therefore, excluding the impact of these elements into the classification. The new approach demonstrated differences in clinical baseline characteristics and both local and distant disease progression[24]. Basal-like 1 (BL1) revealed increased markers of proliferation, and elevated expression of the DNA damage response (DDR) genes. Basal-like 2 (BL2) were characterised by features of basal/myoepithelial origin and activation of growth factor pathways such as *EGF, NGF, MET, Wnt/β*-catenin, and *IGF1R*. The mesenchymal (M) subtype displays activation of pathways involved in epithelial–mesenchymal transition (EMT), cellular differentiation, and growth pathways. Luminal androgen receptor (LAR) tumours are characterised by a high expression of androgen receptor (*AR*) and downstream AR targets, and enrichment of pathways involved in steroid synthesis, porphyrin metabolism, and androgen/oestrogen metabolism[23].

A retrospective analysis from the validation cohort of the TNBC subtype classification presented by Masuda et al showed that the likelihood of pCR with NACT was subtype dependent. BL1 had the highest pCR rate; BL2 and LAR had the lowest. TNBC subtypes demonstrated improved pCR predictions as compared to intrinsic subtypes (basal-like vs. non-basal)[25]. In a retrospective analysis of clinically annotated microarray datasets of BC patients, TNBC type-4 subtyping was not associated with significant differences in pCR in the TNBC subgroup. However, the overall incidence of pCR for the subtypes demonstrates trends similar to those observed in previous studies. BL1 displayed the greatest pCR rate (41%) and LAR and BL2 displayed the lowest (29% and 18% respectively). BL1 patients had significantly higher pCR rates compared with other subtypes (49% vs. 31% p=0.04)[24]. Santonja et.al explored the performance of Lehmann subtypes and their association with pCR in 125 TNBC patients treated with neoadjuvant anthracyclines and/or taxanes with and without carboplatin and their results were consistent with previous reports[26]. The pCR rate for carboplatin containing regimens was highest for BL1 tumours (80% vs 23%, p=0.027). LAR tumours had the lowest pCR rate to all treatments (14.3% vs 42.7%, p=0.045).

3.2.3. Burstein subtypes

Burstein and colleagues applied non-negative matrix factorisation clustering to identify four distinct TNBC subtypes characterised by key molecular features and prognosis: LAR, mesenchymal, basal-like immunosuppressed (BLIS), and basal-like immune-activated (BLIA). BLIS and BLIA showed the best and worst clinical outcomes, respectively. LAR and mesenchymal subtypes revealed significant overlap with Lehmman's classification. Burstein's subtypes based on immune signalling (BLIA, BLIS) revealed a combination of BL1 and BL2 subtypes[27].

3.2.4. FUSCC classification.

Liu et al. developed a classification system based on the transcriptome profiles of both messenger RNAs and long non-coding RNAs to divide TNBC into four distinct clusters. Cluster A: immunomodulatory subtype, Cluster B: luminal androgen receptor subtype (LAR), Cluster C: mesenchymal-like subtype, and Cluster D: basal-like and immune-suppressed (BLIS) subtype. No significant difference in prognosis was found between the four subtypes. Tumours classified as BLIS subtype experienced poorer relapse-free survival (RFS) compared to all other subtypes[28,29]. Further classification of BLIS tumours based on their homologous recombination deficiency (HRD) status[30] showed that high-HRD BLIS TNBCs and low-HRD BLIS TNBCs exhibited distinctive genomic characteristics and prognoses. Patients with tumours defined as low-HRD had a worse prognosis than those in the high-HRD subgroup (5-year RFS of 73% and 95%, respectively, p = 0.002)[29].

3.2.5. Integrative Clusters

Combining GE and DNA copy number analysis within the METABRIC dataset further expanded the taxonomy of breast cancer[31]. Eleven Integrative Clusters (IntClust) with distinctive copy number profiles and clinical outcomes were identified. TNBC are most frequently classified as IntClust 4ER- or IntClust 10. Rueda et al. showed that patients with tumours classified as IntClust 10 (n=222) have a low probability of late relapse (five years after diagnosis); while those classified as IntClust4ER- (n=73) show a persistent and increasing risk of relapse or cancer-related death after 5 years. Classification by immunohistochemistry or intrinsic subtypes did not segregate this risk[32]. The predictive value of IntClust to define response to NACT is yet to be fully established.

3.2.6. Prado-Vasquez classification

Prado-Vasquez et al developed a probabilistic graphical model to classify the cellular component of tumours into four groups based on the 'stem cell hypothesis', defined based upon the grade of development of the cells from which they derived: Luminal (LAR), basal, claudin-high (CLDN-high), and claudin-low (CLDN-low). The sparse k-means method was used to define high or low immune activity, and to classify the tumour as Immune metanode positive or negative. Immune metanode activity was prognostic overall, and particularly in the Luminal group defined by the cellular classification and TNBC type4 -LAR[33].

Combining molecular knowledge with patient management is an increasingly accepted practice across tumour types. In early TNBC, a lack of reproducibility and the absence of a unified approach have led to the continuous use of unselected clinical strategies that remain insufficient. Stable commonalities among the classification methods of molecular subtyping in TNBC suggest the presence of clear biological groups suitable for personalised therapeutic interventions. For instance, luminal-like and mesenchymal tumours are consistently identified across the methods with decent overlap and reproducible outcome data. Moreover, most methods include a measurement of the interaction between tumours and immune response, highlighting the importance of considering this element a key component of the TNBC taxonomy. Overall, these efforts provide the basis to understand how the molecular complexity of TNBC influences outcomes. Considering treatment response as the result of dynamic network interaction, rather than focusing on individual static components, is likely to have more predictive power. But even with reproducible and reliable classification delivering this in a clinical timeframe suitable for neoadjuvant therapy decision-making remains a challenge.

Table 2: Common TNBC Classification Methods *Most prevalent intrinsic subtypes in TNBC listed

Summary Box 1 - Biological and clinical features of TNBC

- **TNBC is characterised by the absence of ER, PR and HER2 expression and is associated with high early response rates to treatment and poor prognosis.**
- **TNBC is a heterogeneous disease with a high level of inter and intra tumour heterogeneity.**
- **Multiple TNBC classifications that split TNBC tumours based on unique molecular features have been described but have yet to be incorporated into routine clinical practice.**

4. Overall approach to the treatment of early stage TNBC

Therapeutic options for early TNBC were traditionally limited to cytotoxic chemotherapy, surgery and radiotherapy. Significant advances in basic and clinical research have led to tangible improvements in the current therapeutic arsenal. Pembrolizumab immunotherapy has now been approved by the FDA for use in combination with chemotherapy for high-risk early-stage TNBC following survival data from the KEYNOTE-522 trial[34]. This has established immunotherapy as a new standard of care in the United States, and it is anticipated to reach clinical practice in other countries in the near future. Similarly, the recent FDA

approval of Olaparib for the adjuvant treatment of high-risk germline *BRCA*(*gBRCA*) carriers following results of the OlympiA trial is expected to reshape clinical practice[35]. These encouraging developments highlight the importance of a personalised treatment approach and focus attention on the unresolved challenges of appropriate patient selection and derived toxicity.

Closing the gap between pre-clinical advances and the clinical setting remains a lengthy and challenging process.

4.1. (Neo)adjuvant Chemotherapy

The effect of polychemotherapy compared with no chemotherapy across all BC subtypes was assessed as part of the 2012 Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis of 32,000 patients. This showed a ~50% reduction in 2-year recurrence and 20-25% reduction in BC[36]. Chemotherapy is particularly important in managing TNBC as these tumours demonstrate a better response as compared to other subtypes of BC and the importance of achieving and optimising early treatment response in these tumours is well recognised.

4.1.1. Anthracyclines

Anthracyclines target cell proliferation pathways by interacting with DNA gyrase and leading to DNA double-strand breaks (DSBs). The ABC trials proved the addition of an anthracycline to taxane and cyclophosphamide improved patient outcomes, with the greatest benefit in high-risk patients; those with lymph node involvement or hormone-negative disease[37]. More recently, a large meta-analysis by Braybrooke et al. found an 18% reduction in 10-year recurrence risk with the addition of anthracycline to taxane chemotherapy, as compared to taxane alone[38]. There are multiple anthracycline-taxane based regimens now in use, with evidence to support one "optimal'' standard of care regimen for TNBC lacking[39].

Anthracycline-free chemotherapy regimens are considered when cardiotoxicity is a concern, and routine use of such regimens for treatment de-escalation is an area of increasing interest[40]. Evidence regarding efficacy as standard treatment for TNBC is conflicting, although a recent meta-analysis has established anthracycline-free chemotherapy to be acceptable for lower risk, early-stage HER2-negative BC[39].

Table 3: Major clinical trials evaluating adjuvant anthracycline-free chemotherapy regimens for patients with stage I-III TNBC

4.1.2. Microtubule Targeting Agents

Taxanes inhibit cell division by stabilising microtubules, preventing depolymerisation, spindle formation, and progression through the cell cycle. Paclitaxel and docetaxel are regularly used to treat early-stage TNBC. An EBCTCG meta-analysis showed the addition of taxane to anthracycline resulted in a proportional reduction in mortality rates of 15–20%[61]. The European Cooperative Trial in Operable Breast Cancer (ECTO) also demonstrated significant improvements in RFS and distant RFS[45]. Although this evidence is not unique to TNBC, these studies provide the strongest evidence to support taxane use in this cohort. BL1 and BL2 tumours appear to derive an increased benefit from this drug class[46].

There are several novel alternatives to traditional taxanes under investigation. Nab-paclitaxel is a solvent-free albumin-bound nanoparticle formulation of paclitaxel. It potentially enables higher intra-tumoural taxane concentrations, better efficacy and improved tolerability. The GeparSepto[47] and ETNA trials[48] showed conflicting results with a significant difference in pCR rates seen only in GeparSepto (Table S2) which may reflect the relative dose intensities used.

Epothilones are a promising alternative to taxanes in development. These novel potent microtubule stabilisers can bypass common resistance mechanisms seen with taxanes, such as drug efflux pumps and β-tubulin. In the early setting, the phase 3 TITAN trial has shown similar efficacy, and reduced rates of peripheral neuropathy, dose modifications and discontinuation with Ixabepilone in comparison with paclitaxel[50].

4.1.3. Platinum salts

The clinical activity of platinum agents has been significantly associated with a DDR vulnerability in both sporadic and *gBRCA*-associated TNBC. Carboplatin is increasingly used in neoadjuvant regimens, improving both pCR and long term outcomes[51]. Please see section 5.1.

4.1.4. Capecitabine

Capecitabine is an oral prodrug of the antimetabolite 5-fluorouracil. Capecitabine is not currently recommended in clinical guidelines for the neoadjuvant or adjuvant treatment of TNBC, though it is selectively used as a post neoadjuvant treatment for residual TNBC. Evidence for use in the adjuvant setting is accumulating, but in most cases studies have not incorporated the molecular features of the TNBC cohort into the planned analysis for response assessment. The recent phase 3 CBCSG-010 trial for unselected patients with TNBC with concomitant use of capecitabine 1,000 mg/m2 and standard anthracycline-taxane adjuvant chemotherapy (ACT) established a significant 5-year disease free survival (DFS) benefit[52]. This is supported by the FINXX trial[53] and the Ye et al. meta-analysis which demonstrated improved DFS and OS with a tolerable increase in toxicity[54].

4.2. Bone modifying agents

Adjuvant bisphosphonates are recommended for breast cancer patients with low-oestrogen status at high risk of relapse to decrease skeletal metastases and improve OS and DFS, as evidenced by the AZURE trial and EBCTCG meta-analysis, both of which included patients of all BC subtypes[55,56]. While the majority of evidence for bone modifying agents in TNBC comes from studies of patients receiving ACT, benefit is also likely to be derived in the neoadjuvant setting[57]. A subgroup analysis of patients receiving neoadjuvant ZA alongside NACT in the AZURE trial led to improved pCR rates[58]. The role of RANK-L remains under investigation. The D-CARE trial of adjuvant denosumab showed no improvement in bone metastases free survival, invasive disease free survival (iDFS) or OS in high-risk early breast cancer. This suggests the mechanisms by which bisphosphonates act against the metastatic potential of BC cells are broader and more sustained than the known effects on bone cell function[59].

Summary Box 2 - Standard of care treatments in TNBC

-Sequential anthracycline-taxane based regimens are considered standard of care.
-Anthracycline-free chemotherapies are considered for lower risk tumours or in patients where cardiotoxicity is a concern.
-Taxane-free chemotherapy or use of an alternative microtubule stabiliser is considered in patients with peripheral neuropathy or taxane hypersensitivity reactions.
-Bisphosphonates are recommended for the treatment of operable breast cancer of all subtypes in patients with low oestrogen states, whether natural or induced. They should particularly be considered in patients at high risk of relapse or treatment-related bone loss.

4.3. Treatment Schedule

4.3.1. Neoadjuvant vs. Adjuvant Chemotherapy

Chemotherapy can be delivered in the adjuvant or neoadjuvant setting with no significant difference in long-term outcomes, as illustrated by the NSABP B-18, EORTC 10902, and IBBGS trials[60-62]. More recently, an EBCTCG meta-analysis demonstrated no significant difference in distant recurrence or death between NACT and ACT but a more frequent local recurrence rate[63]. A TNBC specific meta-analysis suggested NACT is associated with a comparable DFS but worse OS than ACT[64], perhaps explained by patients with higher disease burden being more likely to receive NACT. In this meta-analysis patients that achieved pCR,

had superior OS and DFS compared to those treated with ACT. This evidence does not support the suggestion that NACT promotes cancer cell dissemination[65].

Advantages of NACT include downstaging tumours resulting in increased rates of breast-conserving surgery and associated improved cosmesis and reductions in postoperative lymphoedema. In addition, it allows assessment of treatment response, provides valuable prognostic information[66], guides choice of post-surgical treatment, and allows for ineffective treatment to be ceased to avoid unnecessary toxicity. NACT also provides an ideal platform for translational research, assessment of biomarkers, and genetic testing[67].

The same Anthracycline/Taxane-based regimens are typically used in NACT and ACT. Whether the scheduling of these combinations has any effect on efficacy has been a matter of extensive research. The evidence suggests using taxanes and anthracyclines sequentially increases efficacy and decreases toxicity[68]. There is some evidence to show administration of taxane chemotherapy before anthracyclines is associated with improved pCR rates[69].

4.3.2. Dose-dense and metronomic chemotherapy

There has been increasing interest in personalising treatment schedules to take patient and tumour characteristics into account. Dose-dense NACT is now a widely accepted treatment strategy for high-risk TNBC in order to prevent cancer cell repopulation[70]. It has been consistently shown to improve rates of pCR, breast-conserving surgery, and recurrence in hormone-low BC[71,72]. Although this regimen has not translated into a significant survival benefit[72], this approach should be considered in selected patients with a high disease burden. Dose-dense ACT improves DFS and OS rates in patients with low hormone receptor levels, although this is accompanied by increased toxicity and patients need to be selected carefully[73].

At the other end of the spectrum, metronomic chemotherapy is given at minimum biologically effective dose either continuously or with minimal extended breaks from treatment to reduce severe toxicity. It is thought to have angiogenic, stroma targeting, and immunostimulatory effects[74]. It has been investigated as a single approach as well as being used in combination to intensify standard chemotherapy. It may have a role as a maintenance therapy for high-risk patients or for use by patients who would not otherwise be able to tolerate the adverse effects of standard treatments. The SYSUCC-001 study showed significant improvement in 5-year DFS with 1 year of maintenance capecitabine[75]. The IBCSG 22-00 trial confirmed a 7.9% reduction in the absolute risk of relapse in patients with node-positive TNBC[76] after 1 year of low dose capecitabine and methotrexate maintenance treatment, although no improvement in DFS was observed.

4.4. Assessing Response to NACT

Residual Cancer Burden (RCB) is a prognostic score which classifies tumour response to chemotherapy using a numeric score based on four characteristics of surgical outcome: primary tumour bed dimensions, cellularity fraction of invasive cancer, size of largest metastasis, and number of positive lymph nodes[67]. Four prognostic categories were established (Table 4). It has been shown NACT achieves a pCR in slightly over a third of patients with TNBC and these patients enjoy excellent long term survival outcomes[77]. Higher rates of pCR following NACT are seen in TNBC, as compared to other subtypes, despite the high rate of disease relapse in this cohort. This is believed to derive from poor outcomes in patients with residual chemotherapy-resistant disease[78]. RCB after NACT can accurately predict both event-free survival (EFS) and DFS and is commonly used as a surrogate outcome in clinical trials[79].

Liquid biopsies for circulating tumour DNA (ctDNA) measurement is a promising dynamic approach to assess for residual disease and to predict treatment response in real-time[80]. Fragments of DNA released by apoptosed or necrosed tumour cells can be longitudinally measured in patients' blood samples. Detection of high ctDNA levels at the time of surgery has been associated with reduced DFS and OS rates and clearance of ctDNA during NACT has been associated with improved outcomes across all BC subtypes[81]. Clinical trials that incorporate this approach for patient selection are imminent.

Table 4: Residual cancer burden categories

4.5. Post Neoadjuvant Treatments

Patients with residual disease at surgery are often considered for further systemic therapy. Current treatment options in this setting following NACT include capecitabine and Poly ADP-ribose polymerase inhibitors (PARPi) for g*BRCA* carriers.

The Create-X trial demonstrated that six to eight cycles of capecitabine improved 5-year DFS and OS as compared to no further therapy, especially in the TNBC cohort[82]. In contrast, the GEICAM/2003-11_CIBOMA/2004-01 trial failed to show a statistically significant increase in DFS with the use of eight cycles of adjuvant capecitabine[129]. Of note, a pre-planned analysis of this study showed that the non-basal TNBC cohort derived most benefit from receiving capecitabine[83]. Significant differences in study populations limit direct comparisons between these two studies. Create-X enrolled an Asian population who are known to be highly efficient metabolizers of fluoropyrimidines, all of whom had high-risk pathologically-assessed residual disease. In contrast, GEICAM/CIBOMA accrued patients from Europe and South America, only 80% of whom had residual disease. Meta-analyses on the topic have concluded upon an overall improvement in DFS and OS with capecitabine[84] and opinion from the St Gallen international conference found 87% of experts would offer capecitabine to patients with residual TNBC in the post-neoadjuvant setting[85]. Differences in outcomes on a population level and issues with toxicity have led to capecitabine being offered on a case-specific basis rather than as standard of care[86]. The GEICAM/CIBOMA data indicates that more detailed investigation is needed of exactly which TNBC sub-types would benefit from capecitabine.

Table 5: Major clinical trials evaluating capecitabine in patients with stage I-III TNBC

The OlympiA trial recruited 1836 patients with HER2 negative cancers, 82% classified as TNBC, and showed that 52 weeks of adjuvant Olaparib was associated with a significant DFS improvement in patients with g*BRCA1/2* mutations (3-year iDFS 85.9% for Olaparib vs 77.1% for placebo)[35]. A 32% reduction in the risk of death versus placebo (HR=0.68; 95% CI 0.50-0.91; p=0.0091) led to the recent FDA approval for Olaparib in this setting.

The optimal treatment for residual disease after NACT remains a matter for debate, particularly for g*BRCA* carriers with high-risk TNBC. A direct comparison between adjuvant Olaparib and capecitabine is not available. The theoretical advantage for Olaparib use includes targeting a known tumour susceptibility in a selected population, leading to improved response, and improved tolerability as compared to standard cytotoxics. Interestingly, a phase 2 trial that assessed the value of molecularly targeted postneoadjuvant treatment vs clinician's choice in TNBC patients with residual disease did not demonstrate superiority of this approach[87]. Despite the limitations in regards to the primary outcome, an example was set for biomarker-driven clinical trials and the use of ctDNA in optimising the selection of biomarker-treatment partners. Patient preference and financial issues clearly also need to be considered in this setting.

Summary Box 3 - Key concepts in the current treatment of TNBC

-Chemotherapy can be given in the adjuvant or neoadjuvant setting and the same regimens are typically used. Long-term survival outcomes are similar.

-Advantages of NACT include a rapid evaluation of tumour response, prognostication using RCB scoring, and improved surgical outcomes.

-RCB is strongly associated with long term outcomes in TNBC

-Patients with TNBC who are at increased risk of relapse after chemotherapy in the neoadjuvant setting benefit from adjuvant capecitabine. Patients in the g*BRCA* subgroup benefit from PARP inhibitors.

-Sequential liquid biopsies to assess ctDNA level represents a possibility for monitoring treatment response in real-time.

5. Targetable molecular pathways

Much progress has been made to define and treat TNBC according to aberrations on the molecular level, although the derivation and use of biomarkers to select patients for specific treatments has been somewhat lacking. In order to make further progress, the identification of predictive biomarkers must be a central focus of our research and once secure, used to guide and to select patients most likely to derive benefit from targeted treatments.

Tables S3, S4 and S5 summarise ongoing trials contributing to the use of molecularly targeted treatment for early TNBC

5.1. DNA damage response (DDR)

TNBCs are frequently deficient in DDR pathways and exhibit high chromosomal instability[7,88]. Repair of DNA double-strand breaks (DSBs) relies on the homologous recombination (HR) pathway. Dysfunctional activity of genes involved in this pathway compromises the ability of cells to mend DSBs, thereby inducing Homologous Recombination Deficiency (HRD)[89].

HRD can occur via numerous mechanisms, all resulting in similar phenotypic and genotypic features to those of *BRCA* mutant tumours; an observation that has been termed '*BRCA*-ness'. Phenotypic and molecular similarities between *BRCA*-associated BC and sporadic TNBC have led to the application of similar therapeutic interventions in both groups. In patients with *BRCA* mutations and *BRCA*ness features, a compromised DDR pathway facilitates increased sensitivity to drugs such as platinum and PARPi, based on the concept of synthetic lethality[90].

Approximately 10-20% of TNBC harbour g*BRCA* mutations, and 70% of *gBRCA1* and 16% of *gBRCA2*-associated tumours are classified as TNBC[91]. Somatic *BRCA* mutations are uncommon in sporadic TNBC[6,18,29]. *BRCA*1 and 2 mutations, and hypermethylation of *BRCA*1 promoter, only account for some TNBCs that exhibit functional evidence of HRD. Around 40% of BCs are identifiable as HRD in the absence of these changes[92]. Dysfunctional *BRCA* pathways are frequently enabled by other mechanisms, for example, *RAD51* and *PALB2* mutations can confer a *BRCA*-ness phenotype[92].

5.1.1. Therapeutic approaches

5.1.1.1. Platinum agents

The cytotoxic activity of platinum is mediated by the formation of platinum–DNA adducts that interfere with DNA replication and transcription, activating DNA-damage recognition and repair, cell-cycle arrest, and apoptosis.

Platinum-containing regimens have not been regarded as standard of care in the treatment of TNBC in most guidelines to date. Several trials have investigated the addition of platinum agents to standard chemotherapy for this subgroup based on the potential increased susceptibility of TNBC to DNA-damaging compounds[25] (Table 6). Improved pCR rates with the addition of carboplatin have been a consistent finding, with confirmed EFS benefit in two large randomised studies, GeparSixto and BrighTNess[93–99]. These results have led to the inclusion of carboplatin within neoadjuvant regimens for high-risk TNBC in an American Society of Clinical Oncology guideline[99].

Combining carboplatin with anthracycline/taxane NACT increases haematological and gastrointestinal toxicity, which in turn has implications for patient selection. Predictive biomarkers to identify those patients deriving the most benefit from the addition of platinum, for example, *gBRCA* mutations, have been investigated. Single-agent cisplatin has shown conflicting results in *BRCA* carriers[100]. The PARTNER

(NCT03150576) trial includes a cohort of *gBRCA* mutated patients[101] and will help to elucidate the effect of platinum and PARPi in this subgroup.

There is currently no routine indication for platinum agents in the post-neoadjuvant setting. The EA1131 study (NCT02445391) was closed early as neither cisplatin nor carboplatin were able to demonstrate non-inferiority or superiority over capecitabine, and toxicity rates were higher[102].

Table 6: Major interventional clinical trials involving platinum agents in patients with stage I-III TNBC

5.1.1.2. PARP inhibition

Poly ADP-ribose polymerase (PARP) activity is crucial for maintaining the correct fork speed and fidelity of DNA synthesis. PARP1 is involved in the response to single-strand DNA (ssDNA) damage and maintains genome integrity via base excision repair. PARP1 is also a critical early event for DNA DSBs repair activation and regulation of resection[161]. PARP inhibition causes replication stress, induces ssDNA breaks and affects the normal regulation of p53 and its downstream effectors[106]. In tumours that have deficiencies in the HR pathway, the accumulation of DSBs originating from primary ssDNA breaks leads to cell cycle arrest and death[107].

Robust evidence now supports the efficacy of single-agent PARPi in BC patients with g*BRCA* mutations who have received prior chemotherapy[108,109]. A variety of PARPi and combinations have now been explored in both patients with g*BRCA* mutations and sporadic (non-*BRCA*) TNBC in the early setting.

Evidence to date for the use of **Olaparib** is promising, both as monotherapy and in combination with chemotherapy, immunotherapy, or radiotherapy. In the neoadjuvant setting, Olaparib was given as monotherapy in 32 patients with unselected TNBC for up to 10 weeks before chemotherapy[110] with overall objective response rate 56.3% vs 51.9% among patients not harbouring *gBRCA1*/2 or germline *PALB2* mutations. A numerical enrichment of somatic HR mutations and *BRCA*1 methylation in the responding group suggests favourable activity of Olaparib here. Other trials in the neoadjuvant setting combine Olaparib with chemotherapy. GeparOLA included patients with HER2-negative BC and HRD, delivering paclitaxel with Olaparib or carboplatin followed by epirubicin and cyclophosphamide[111]. No formal testing between the arms was planned but increased benefit from Olaparib was observed in young (<40 years) and HR-positive patients. In the TNBC subgroup, pCR rate was 56.0% with olaparib and 59.3% with carboplatin. PARTNER is a phase 3 trial that assesses the addition of Olaparib to neoadjuvant platinum-based chemotherapy in the treatment of TNBC and g*BRCA* derived tumours. Preliminary safety results show that the combination of Olaparib and platinum has an acceptable and manageable toxicity profile[112]. In the I-SPY2 trial, research arm patients received Olaparib and Durvalumab with paclitaxel then doxorubicin and cyclophosphamide [113] which increased pCR in the TNBC group (27%–47%), Immune-rich tumours had greater sensitivity to this treatment. The adjuvant phase 1 RadioPARP trial for patients with inflammatory, locoregionally advanced or mTNBC, or patients with residual disease after surgery for TNBC, sought to evaluate safety and dosing for Olaparib in combination with radiotherapy[114]. Olaparib was escalated to the maximum target dose of 200 mg twice daily with no dose-limiting toxicity.

Talazoparib has been reviewed in the neoadjuvant setting as monotherapy and in combination with chemotherapy. TALA was a pilot study that recruited 20 patients with operable BC and a *BRCA* mutation to receive Talazoparib monotherapy for 6 months[115]. Despite the small sample size, this trial showed an encouraging pCR rate of 53% and RCB-0/I of 63%, with a manageable safety profile. In the I-SPY2 trial, Talazoparib combined with irinotecan for HER2 negative patients had limited activity beyond that seen with standard treatment[116].

Veliparib has also been evaluated in the neoadjuvant setting in the I-SPY2 trial[117]. The addition of Veliparib to carboplatin containing chemotherapy increased pCR rate in the TNBC group from 26% to 51%. This combination was further assessed in the phase 3 BrighTNess trial in 634 patients with TNBC[98] where no additional benefit for Veliparib above that achieved by adding carboplatin, regardless of *BRCA* mutation status, was found. A key limitation of this study is the low dose of Veliparib, less than half of that used in

the BROCADE-3 study in the advanced disease setting[118]. Veliparib has been combined with radiotherapy for inflammatory or locoregionally recurrent TNBC which resulted in significant local toxicity[119].

Both Talazoparib and Olaparib are effective as monotherapy in patients that carry g*BRCA* mutations. Given the low dose of Velaparib used in the BrighTNess trial, and taking into account individual PARPi differences in PARP trapping capacity, the potential summative benefit from the addition of platinum to PARPi cannot be excluded. This encourages further investigation into the role of other PARPi such Olaparib and Talazoparib, and the great potential for combination therapy, as demonstrated by ongoing trials in Table S3.

5.1.1.3. Other DDR agents

The ATR inhibitor **Ceralasertib** (AZD6738) is being investigated as monotherapy in chemotherapy-resistant TNBC as part of a pre-surgical window of opportunity and post-surgical biomarker study (NCT03740893, PHOENIX), reviewing the change in mean proliferation index between baseline and post-treatment. PARTNERING is a phase 2 sub-study for the PARTNER trial that offers Durvalumab in combination with AZD6738 to patients with evidence of residual disease after completion of NACT and before surgery. WEE 1 inhibitors have not yet been reviewed in the early TNBC setting.

Table S3 summarises the major incomplete clinical trials involving DDR agents in patients with stage I-III TNBC.

Summary Box 4 - DNA damage response: treatment strategies

-There is strong evidence to support the addition of platinum agents to NACT to improve patient outcomes, especially in high risk and g*BRCA* carriers.
- Improvements in pCR and EFS rates with platinum chemotherapy combinations need to be balanced against additive chemotherapy toxicities.
-PARP inhibition causes replication stress, induces ssDNAs breaks and affects the normal regulation of p53 and its downstream effectors.
-Encouraging evidence supports the efficacy of single agent PARPi in BC patients with g*BRCA* mutations who have no prior chemotherapy exposure.
-The group of patients with TNBC most likely to benefit from PARP inhibition in the neoadjuvant setting is yet to be established.
- Olaparib improves DFS in g*BRCA* carriers with high risk HER2 negative disease following neoadjuvant or adjuvant chemotherapy.

5.1.2. Predictive biomarkers of DDR agents

5.1.2.1. *BRCA* mutations

The predictive value of both g*BRCA* and somatic *BRCA* mutations for response to platinum and PARPi has been validated in large clinical trials that include patients with ovarian and metastatic BC[109,115]. The role of *BRCA* status as an independent predictive biomarker for the TNBC population in the neoadjuvant setting is still unclear with studies showing conflicting results. In a secondary analysis of the GeparSixto trial (*n=50)*[120], *gBRCA* mutations were predictive of higher pCR rates and carboplatin did not increase this further. In the CALGB 40603 trial, pCR rates in patients with g*BRCA* mutations were similar to the overall population, and this outcome was not altered by the addition of carboplatin[121].

BRCA1/2 mutation carriers with TNBC subtype in the I-SPY 2 trial were significantly more likely to achieve a pCR than non-*BRCA* TNBC (predicted pCR of 75% vs 29%)[122] and a greater response was seen for patients with a *BRCA*-ness signature[117]. Subgroup analysis of the BrighTNess trial did not show a difference in pCR rate based on *BRCA* status[46]. However, in the GeparOcto trial[123] g*BRCA* mutation carriers gained greater

benefit from platinum (68.1% vs 45.7%, p=0.005), particularly in the TNBC subgroup (74.3% vs 47%, p=0.005).

In the PETREMAC trial, in which patients received olaparib monotherapy before chemotherapy, pathogenic mutations (germline or somatic) in the HR pathways and/or *BRCA1* promoter methylation were associated with Olaparib overall response (OR) 88.9%[124]. Although pCR rates in the GeparOLA trial for *gBRCA1*/2 carriers were significantly higher than in non-carriers (62.7% vs 41.3%, P=0.047), exploratory analysis revealed no difference between treatment arms if somatic or germline *BRCA1*/2 mutation were detected[125].

5.1.2.2. HRD by gene sets analysis and functional assays

Several attempts to simplify and systematically identify common molecular changes associated with defective HR have been published. The evaluation of DNA damage repair-related genes by either gene expression or by the presence of mutations has shown a positive association with response. Confirmation of the predictive value of these individual efforts has not always been accomplished given the underlying heterogeneity of some of these variations. (**Table 7)**.

5.1.2.3. HRD by genomic scars and mutational signatures.

The detection of mutational signatures uniquely identifying patterns of defective HR repair is the subject of several studies. Vollebergh et.al assessed whether array comparative genomic hybridisation patterns could predict the benefit of intensified carboplatin-based chemotherapy[126]. A HRD score defined by an unweighted sum of loss of heterozygosity, telomeric allelic imbalance, large-scale transition, and *BRCA*1/2 mutations has been tested in TNBC treated with platinum, and used to aid patient selection in PARPi trials[127,128]. In the absence of g*BRCA* mutations, a high HRD score was associated with higher pCR rates irrespective of the use of carboplatin. Microhomology-mediated indels, HRD index, single base substitution signature 3, rearrangement signature 3 and 5, and genomic instability markers of HRD are aggregated into the HRDetect score[92]. The prognostic value of HRDetect has been demonstrated in two retrospective clinical cohorts and further evaluation of its predictive power in randomised clinical trials is awaited.

HRD is yet to be used to guide in the clinical management of TNBC despite its theoretical significance. The absence of a standardised definition of HRD beyond g*BRCA* mutation and the lack of prospective clinical trial data, currently limit clinical utility.

Table 7: HRD related biomarkers and its association with treatment response.

5.1.2.4. Tumour mutational burden

An increased number of tumour mutations could be correlated with an enhanced response to drugs causing DNA damage. For example, somatic hypermutation was shown to be an independent factor for estimating the risk of platinum sensitivity in high-grade serous ovarian cancer (OR=3.616, p=0.002)[132]. A higher tumour mutational burden (TMB) has been observed in BCs that harbour DDR gene mutations[133], although correlation with response to platinum is not yet established. Contrastingly, in the PETREMAC trial, no difference in TMB was observed between responders and non-responders, or *BRCA* carriers versus non-carriers[107].

Summary Box 5 - DNA damage response: biomarkers

-The role of *BRCA* status as an independent predictive biomarker among the TNBC population in the neoadjuvant setting is unclear
-Overall, alterations in DNA damage repair-related genes by either gene expression or presence of mutations has shown a positive association with response to NACT and/or PARPi.
-Mutational signatures predictive of *BRCA1/BRCA*2 deficiency or a `*BRCA*-ness status` have shown a trend to positive association with response to platinum chemotherapy. However, these

results are signature specific and should be considered preliminary. Data from randomised clinical trials that prospectively assess the value of these biomarkers is awaited.
-Higher TMB has been observed in BC tumours that harbour DDR gene mutations. Correlation with response to platinum agents is not yet established.

5.2. Immune response

Although BC is largely considered an immune quiescent cancer type[134], increasing evidence suggests that a range of tumour immunogenicity is present. TNBC is characterised by increased immune activation and wide immune heterogeneity compared to other BC subtypes[135].

5.2.1. Therapeutic approaches

Tumours evade detection and eradication by the immune system through the dysregulation of pathways controlled by immune checkpoints. Immunotherapy harnesses the patient's immune system to target malignant cells using Immune checkpoint inhibitors (ICI), Chimeric antigen receptor T cells or cancer vaccines. ICIs release the immune system from tumour-induced inhibitory signals, allowing an effective anti-tumour response. They include monoclonal antibodies (mAbs) against cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), programmed cell death-1 (PD-1), and programmed cell death ligand-1 (PD-L1).

5.2.1.1. Monoclonal antibodies against PD-1

Pembrolizumab is the most well-established and successful anti-PD-1 ICI in operable TNBC. The addition of Pembrolizumab to NACT has shown increases in pCR rate across several RCTs including the KEYNOTE-173 and I-SPY 2 trials[136,137]. These successes led to the landmark phase 3 KEYNOTE-522 trial which has culminated in the FDA approval for use of Pembrolizumab in high-risk early-stage TNBC, the first regulatory approval for an immunotherapy agent in this setting. Pembrolizumab is now considered a standard of care treatment in the United States for patients fitting trial eligibility criteria.

KEYNOTE-522 evaluated neoadjuvant Pembrolizumab in combination with carboplatin/paclitaxel and anthracycline-based NACT, and then adjuvantly as monotherapy, in high-risk early TNBC. pCR rate improved by 7.5% (95% CI: 1.6% to 13.4%) with the addition of Pembrolizumab, and after a median follow up of 39.1 months, 36-month EFS improved from 77% to 85% (HR: 0.63; 95% CI, 0.48 to 0.82; P<0.001). OS data remains immature at the time of analysis[34]. High-risk patients derived the greatest benefit with higher absolute improvements in pCR in stage III and node-positive disease. There are some limitations to this study. With this trial design, it is not possible to elucidate the relative contributions of the neoadjuvant and adjuvant treatment phases on these EFS results. Concern has been raised at the rate of serious adverse events (77% incidence of grade ≥3 events in the immunotherapy group), and immunotherapy related adverse effects (irAE) (affecting 33.5% of patients on this trial) due to their protracted nature. It is therefore imperative to detect predictive biomarkers to facilitate the selection of patients likely to derive the most benefit from immunotherapy and treatment de-escalation strategies. No predictive biomarkers were identified on this trial. Improvement in pCR rate was seen regardless of PD-L1 status[138]. Patients on the Pembrolizumab arm that achieved pCR derived a modest survival benefit (approximately 2%), as compared to 10% in the cohort of patients with residual disease at surgery. This suggests that the value of adjuvant Pembrolizumab as a monotherapy may be small in the group who achieved pCR. Removal of the adjuvant portion of treatment based on response at surgery could represent a potential treatment de-escalation strategy that requires further exploration.

5.2.1.2. Monoclonal antibodies against PD-L1

Atezolizumab, **Durvalumab** and **Avelumab** are the most established anti-PD-L1 ICIrs being investigated in operable TNBC, although results from trials have been inconsistent. pCR rate improved from 41% to

58% with the addition of Atezolizumab to anthracycline/taxane-based chemotherapy in Impassion031[49]. Secondary endpoints (EFS, DFS and OS) are expected later this year, however, this trial is not powered to show survival differences. The phase 3 NeoTRIPaPDL1 trial failed to show a significant pCR advantage with the addition of Atezolizumab to neoadjuvant carboplatin and nab-paclitaxel[140], although EFS was the primary endpoint and this data is not yet available. These incongruent results are likely to reflect the higher risk patient population in NeoTRIPaPDL1 and the difference in the chemotherapy backbone. Results from the TONIC trial suggest anthracycline chemotherapy, used in Impassion031, leads to a potentiation of the effects of immunotherapy[141]. These insights should inform the choice of chemotherapy backbone in the design of future immunotherapy trials.

GeparNUEVO assessed Durvalumab in addition to anthracycline/taxane-based NACT. This showed a non-significant 9% improvement in pCR rate. Improvements in 3y iDFS and 3y OS were also seen, though this trial was not powered to definitively assess long term survival differences. An underpowered subgroup analysis showed a particular benefit in patients who received Durvalumab alone for two weeks prior to NACT, suggesting immunological interactions with priming in this window phase[142,143]. While the small patient cohort included in GeparNUEVO has comulated in statistically non-significant pCR and iDFS benefits, the results are similar to those from KEYNOTE-522. This is despite lacking a platinum agent and an adjuvant treatment phase. These represent potential treatment de-escalation avenues that could benefit from further exploration. Discrepancy between the magnitude of benefit for pCR rate and survival seen across both trials suggests pCR to be a poor surrogate marker for long term survival in immunotherapy trials in operable TNBC. Published and ongoing trials of ICI have been summarised in **Tables 8 and 9**.

The use of ICIs in TNBC is an area of active research, although it is at an early stage, and long-term outcome data remain immature for the majority of the neoadjuvant trials. Concern regarding the use of pCR as a primary endpoint upon which to grant regulatory approval for neoadjuvant Pembrolizumab was cited by the FDA, and long-term survival data is of particular interest[144]. There is a paucity of data available to guide use of pembrolizumab in the adjuvant or post-neoadjuvant setting, particularly in combination with agents such capecitabine or Olaparib used in more contemporary practice. This represents a challenge when adopting Pembrolizumab as standard of care treatment and results of trials investigating these issues are highly anticipated.

Table 8: Major neoadjuvant trials of immune checkpoint inhibitors in patients with stage I-III TNBC

Table 9: Major adjuvant trials of immune checkpoint inhibitors in patients with stage I-III TNBC. Ongoing trials evaluating PARP inhibitors in combination with immunotherapy can be found in supplementary table 3.

5.2.1.3. Cancer Vaccines

Cancer vaccines utilise tumour associated antigens to stimulate CD4+ and CD8+ T cells, inducing the patient's immune system to target cancer cells that were previously successfully evading immune suppression. They have yet to show success in late-stage clinical trials or to receive regulatory approval for TNBC. Clinical trials evaluating cancer vaccines in non-metastatic TNBC are listed in **Table S4**.

Summary Box 6 - Immune response: treatment strategies

-Immunotherapy is of particular interest in TNBC due to the higher degree of immune activation seen in comparison to other BC types.
-TNBC is a heterogeneous disease that exhibits various degrees of immunogenicity.
-Several early stage BC trials have established PD-1 and PD-L1 ICIs as a promising treatment option in combination with chemotherapy.

- Pembrolizumab has been granted FDA approval in the neoadjuvant setting for high-risk early-stage TNBC in combination with chemotherapy and to continue as monotherapy in the adjuvant setting (KEYNOTE-522).

5.2.2. Predictive biomarkers of ICI response

5.2.2.1. PD-L1

PD-L1 expression is higher in TNBC compared with non-TNBC[136] and quantification is currently performed using five distinct FDA-approved companion diagnostic tests across tumour types. Variety in assays, scoring systems, and cut-off values renders the interpretation of its predictive value challenging[140]. Increased pCR rate in PD-L1+ early-stage TNBC is seen, but rather confusingly, ICI benefit independent of PD-L1 status has been consistently described[138,143,148,149] In the GeparNUEVO trial, pCR rate was increased in PD-L1+ tumours in all therapy groups but PD-L1 did not predict ICI response[143]. Similar results were observed in the KEYNOTE-522 and Impassion 031 trials.

5.2.2.2. Tumour mutational burden

High tumour mutational burden precipitates enhanced immunogenicity by increasing the number of tumour antigenic peptides or neoantigens that can be recognised by T-cells[150]. Based on this hypothesis, high TMB has been correlated with an increased response to ICI[151,152] independently of PD-L1 expression[153]. The FDA granted accelerated approval of Pembrolizumab as monotherapy for advanced tumours that exhibit high TMB (defined as >=10mut/Mb) in 2020[154]. More recently, it has been shown that the association of TMB with response to ICI relies on a positive correlation between CD8+ T-cell level and neoantigen load, and differs across tumour types[131].

The role of mutational load as an independent predictive biomarker of ICI response is yet to be defined in TNBC[155] due to limited data availability and differences among TMB quantification methods. In the GeparNUEVO trial, TMB was higher in patients with pCR (median 1.87 versus 1.39 mut/MB), and both continuous TMB and immune GE profile independently predicted pCR. In comparison, no difference in pCR rate was observed in patients with high TMB who received ICI when compared to other targeted therapies in the ARTEMIS trial (NCT02276443).

5.2.2.3. Tumour infiltrating lymphocytes (TILs)

Both intra-tumoural TILs (iTILS) and stromal TILs (sTILs) have prognostic and predictive roles in the treatment of early TNBC, and have also been evaluated in this setting as a biomarker of immunotherapy response. In the GeparNUEVO trial[141], sTILs prior to therapy predicted a higher pCR rate overall, and in both therapy groups, but were not predictive of Durvalumab response. The increase in iTILs in post-window samples compared with pre-therapeutic samples was predictive of pCR, yet the treatment interaction test did not reach significance (P = 0.085). High TILs were significantly associated with Olaparib response in the PETREMACT trial[110]. Criscitiello et al used a LASSO penalised regression model to develop a 4-gene signature to predict high and low TILs after NACT. High TILs signature was associated with improved long-term outcomes independent of pCR[154]. Overall, increased TILs are associated with a more favourable response to NACT and improved long-term outcomes[156,157].

5.2.2.4. Immune signatures

GE immune signatures have been extensively used to describe profiles of immune infiltration and immune cell type that impact on the prognosis of many tumour types including TNBC[158-161]. Few studies have tested the value of GE immune signatures in the prediction of chemotherapy response in the early setting of TNBC. Sharma et al[162]. evaluated the performance of a DNA damage immune response signature and sTILs as prognostic markers in patients with TNBC treated with adjuvant doxorubicin and cyclophosphamide. in the

SWOG 9313 trial. DDIR was associated with improved OS and DFS, and was moderately correlated with sTILs density (>= 20% v, <20%). Lv et al. identified CXCL9 and CXCL13 as prognostic biomarkers in TNBC using network analysis. Further testing in two neoadjuvant data sets confirmed its predictive value in the response to chemotherapy[163]. Exploratory analysis of the GeparNUEVO trial revealed that predefined TIL and IFN-gamma signatures were associated with increased pCR rate, without specificity for Durvalumab response. The expression of six genes required for immune cell function were significantly correlated with pCR and showed a positive test for interaction with Durvalumab plus NACT[164]. Further evaluation of the interactions between tumour and immune system, as well as its architectural heterogeneity, will provide a more accurate estimation of the individual predictive potential to be derived from immune signatures.

5.2.2.5. Microsatellite instability status

Pembrolizumab monotherapy received FDA approval in 2017 for the treatment of advanced mismatch repair deficient solid tumours[165]. Although only a small proportion of breast cancers are defined as Microsatellite Instable, tumours with defects in the mismatch repair pathways are known to have highly upregulated expression of multiple immune checkpoints and increased sensitivity to ICI[166]. The introduction of new strategies to facilitate the identification of this biomarker in a low frequency cohort like TNBC remains a challenge.

Summary Box 7 - Immune response: Biomarkers

- **Response to ICIs appears to be independent of PD-L1 status in early TNBC.**
- **High TMB has been correlated with an increased likelihood of response to ICI, particularly in tumours where CD8+ T-cell levels are positively correlated with neoantigen load.**
- **The role of mutational load as an independent predictive biomarker of ICI response is yet to be defined in TNBC.**
- **Increased TILs are associated with a more favourable response to NACT and long-term outcomes.**
- **Modest positive association of GE immune signatures with ICI response have been reported.**
- **The interaction between TMB and GE immune signatures** has been **shown to be as a promising independent predictor of pCR.**
- **The dynamics of immune activation after treatment are strongly associated with long term outcome, independently of response rate.**
- **Tumours with defects in the** mismatch repair **pathways are known to have highly upregulated expression of multiple immune checkpoints and increased sensitivity to ICI.**

5.3. PIK3CA/AKT1/PTEN pathway

Dysregulation of the PI3K/AKT/mTOR pathway is often observed in TNBC[18,29], and remains a promising target for the future treatment of this BC subtype. Pathway activation is predominantly via *PIK3CA* mutations (~9-18%), loss of *PTEN* (~35%) or *INPP4B* (~30%), and amplification of *PIK3CA (~43%)*. The frequency of PI3K/AKT/mTOR pathway activation and its spectrum varies by TNBC subtype[18,29], and is strongly associated with the LAR subtype across classifiers.

5.3.1. Alpha-specific PI3K inhibitors

In unselected TNBC, response to PIK3CA inhibitors remains low. The BELLE-4 study evaluated the efficacy of **Buparlisib** in the locally advanced setting for patients with HER2 negative BC in combination with paclitaxel versus placebo and observed no benefit from PIK3CA inhibition[167]. Worse outcomes were observed in the TNBC cohort treated with the PIK3CA inhibitor and lack of benefit was independent of *PIK3CA* mutation or PTEN loss by immunohistochemistry[167]. Shorter treatment duration in the buparlisib

arm due to adverse events, and longer progression free survival (PFS) in the placebo arm than anticipated, are possible explanations for the worse outcomes in this subgroup. The global lack of activity is possibly due to inadequate patient selection and the absence of an accurate biomarker. Parallel pathway activation could also explain a resistance mechanism that requires addressing.

5.3.2. AKT inhibitors

Ipatasertib was reviewed in the neoadjuvant setting in combination with paclitaxel for TNBC patients in the FAIRLANE trial[168]. Adding ipatasertib did not significantly increase the pCR rate compared with paclitaxel alone and this effect was independent of *PIK3CA/AKT1/PTEN* or *PTEN* low status. Complete clinical response was absent in the placebo-treated group in patients with tumours defined as LAR subtype, but was observed in 50% of those treated with ipatasertib. This difference was not evident in pCR rates. Elevated immune scores were more strongly associated with improved outcomes in paclitaxel-treated compared to ipatasertib-treated patients, highlighting the key interaction with the immune system. All ipatasertib-treated patients with low immune scores and complete clinical response had *PIK3CA/AKT1/PTEN*-altered tumours **MK2206** has been trialled in the neoadjuvant setting in the I-SPY2 trial for stage 2-3 BC of any subtype[169]. Patients received paclitaxel chemotherapy with or without MK2206, then AC. pCR for the TNBC group was 40.2% with MK2206 vs. 22.4% without. Following assessment for biomarkers in the AKT pathway in the TNBC subgroup, higher levels of phosphorylated AKT and its substrates were paradoxically associated with reduced response to MK-2206.

5.3.3. MTOR inhibitors

Everolimus has been reviewed in the neoadjuvant setting for patients with TNBC in combination with cisplatin and paclitaxel[26], and in combination with docetaxel, 5-fluorouracil, epirubicin and cyclophosphamide[170]. No improvement in response rate has been demonstrated.

The exact contribution of drugs targeting the PIK3CA/AKT1/PTEN pathway in early TNBC as not yet been defined. The complexity of the immune microenvironment and parallel molecular alterations can obscure an accurate estimation of clinical benefit if they are not both in some way accounted for. It is important to trial these therapies in a way that reduces these confounders and separates the TNBC subtypes to determine their individual response. Current approaches include combining alpelisib with nab-paclitaxel in the neoadjuvant setting (NCT04216472) for anthracycline refractory TNBC with PIK3CA or PTEN alterations, with exploratory objectives to assess biomarkers of response and resistance to alpelisib and nab-paclitaxel combination.

Table S5 summarises ongoing trials that target this pathway in early TNBC.

5.4. AR pathway

AR expression is found in approximately 10-35% of TNBCs as detected by immunohistochemistry [171,172]. The LAR molecular subtype derived from GE accounts for 20-40% of TNBC and is characterised by the activation of AR, ER, prolactin, and ErbB4 signalling. Tumours defined as LAR subtype typically contain a higher number of PIK3CA mutations and pCR rate following NACT is significantly lower compared to other subtypes.

There is a paucity of data for drugs targeting the AR pathway in the early TNBC setting. **Enzalutamide** has been trialled as monotherapy[173], and in combination with PIK3CAi in the advanced setting with modest benefit[174]. Other AR pathway targeted drugs, for example, **Abiraterone** and **Bicalutamide**, have been reviewed in the advanced setting with modest results[175,176]. Although overall benefit remains limited, it is unclear if this derives from inadequate patient selection or analogous pathway activation. Results from four trials in the early TNBC setting are highly anticipated.

Table S5 summarises ongoing trials that target this pathway in early TNBC.

5.5. Receptor tyrosine kinase family

5.5.1. HER2

Approximately 35% of TNBC as defined by immunohistochemistry could be classified as HER2-low[178]. Somatic ERBB2 mutations occur in approximately 3% of TNBC[6], and a subset of TNBC tumours are classified as HER2 enriched by gene expression. This biological heterogeneity has expanded therapeutic opportunities in this population of patients. In an exploratory analysis of a cohort of the I-SPY2 trial, activation of HER2-EGFR was identified as a positive predictor of pCR in 49 TNBC patients treated with a pan-HER inhibitor[177]. A significant correlation between response to HER2 inhibition and HER2 pathway activation has been demonstrated in TNBC cell lines[179].

Neratinib has been investigated in the neoadjuvant setting for high-risk clinical stage II or III BC. The pCR rate overall in the I-SPY 2 trial was 37.5% in the neratinib arm, and among patients demonstrating phosphorylation of *HER2* or *EGFR* (i.e., biomarker-positive for *EGFR* Y1173 or *ERBB2* Y1248), it rose to 63%[180]. Encouraging results in the HER2-low–expressing refractory BC setting with **Trastuzumab Deruxtecan** *(*OR 37%)[181] and **Trastuzumab Duocarmazine** (OR 40%)[182] now require translation into the early setting. These trials illustrate the importance of identifying patients categorised as TNBC who are more accurately defined as HER2 low. **(Table S5)**

5.5.2. VEGF

VEGF promotes angiogenesis, invasion, and increases vascular permeability, and is an essential element in TNBC formation, progression, and metastasis. VEGF-A expression is higher in TNBC compared with other BC subtypes[183], and enhanced angiogenic potential is associated with poor prognosis in BC[184]. Targeting of *VEGF* has been extensively tested in TNBC, but no clear predictive biomarkers of treatment response have been identified.

Trials targeting VEGF in the neoadjuvant TNBC setting have shown disappointing results to date with no difference in DFS or OS. The addition of **Bevacizumab** significantly increased the rate of pCR among patients with Her2 negative disease in some studies[185-188]. The ARTemis and GeparQuinto trials reported increased benefit primarily in the TNBC subgroup. In the adjuvant setting, the BEATRICE trial added Bevacizumab to anthracycline and/or taxane-based chemotherapy[96] and no difference in iDFS or OS between treatment groups was found. The underlying reason for the lack of treatment effect with these drugs is poorly understood. It is possible that a fundamental flaw in either the drug or the signalling pathway is being overlooked. Attempts to overcome drug resistance using novel agents and combinations are ongoing (**Table S5**).

5.5.3. FGFR

The fibroblast growth factor receptor family includes *FGFR1-4*. Signalling through this pathway regulates cell survival, proliferation and differentiation. Genes that encode for these receptors are amplified in ~10% of BC[17]. Although *FGFR1* is the most frequent genomic alteration in all subtypes of BC, amplification, and overexpression of FGFR2 is more frequently observed among TNBC (~4%). Basal BC with elevated *MET* and *FGFR1* signatures is associated with poor relapse free survival[291]. The interplay between MET and FGFR has been shown to regulate cancer stem cells in mesenchymal subtypes[292].

Trial data in this setting is limited to a small number of studies that do not select for TNBC, but in which some response to this target has been seen. It seems likely that the correct biomarker has not yet been identified. Ongoing trials for this target in the neoadjuvant setting include a window of opportunity trial combining Lenvatinib and Pembrolizumab (NCT04427293).

5.5.4. EGFR

EGFR dysregulation is frequently reported in TNBC[191] and enrichment for this pathway signalling is predominantly observed in BL2 tumours[192]. In contrast to EGFR mutations, EGFR amplification is a relatively frequent event (11% vs 23% respectively)[193,17] and is considered an independent prognostic factor

for poor disease-free survival[194]. Several attempts to target this pathway with tyrosine kinase inhibitors and mAbs in the context of mTNBC have been pursued without success. A limited number of trials have used these therapies in the early setting.

Cetuximab has been trialled in combination with neoadjuvant docetaxel in a pilot phase two study including stage II-IIIA TNBC[195]. The pCR rate was 24% [95% CI: 7.3–40.7] and the pre-therapy ratio between CD8+ and FOXP3+ TILs equal or higher than 2.75 was predictive of pCR (43% versus 0%). **Panitumumab** and the EGFR/HER2 inhibitor **Lapatinib** failed to demonstrate additional benefit in the advanced setting independent of EGFR activation[196,197]. The paucity of accurate biomarkers predictive of sensitive patients has led to unsatisfactory outcomes and limited clinical utility despite increasing evidence for EGFR as the driver of tumorigenesis in some TNBC.

Table S5 summarises ongoing trials that target these pathways in early TNBC.

5.6. Other oncogenic targets

Inter-chromosomal rearrangements causing *NTRK* gene fusions can result in constitutive activation of TRK proteins which act as oncogenic drivers through activation of cellular growth pathways. Results from early phase trials that include advanced NTRK fusion-positive solid tumours support the use of **larotrectinib** and **entrectinib** in this subgroup[198,199]. *NTRK* gene fusions occur in low frequency (~0.3%) among all solid tumours[199], however, a high prevalence is observed in a subgroup of TNBC[16]. The *ETV6-NTRK3* gene fusion is frequently found in human secretory breast carcinoma[16], and although the vast majority of these breast tumours are treated with local treatments, targeting TRK signalling remains an option for cases of locally advanced disease.

Trop-2/TACSTD2 is a calcium signal transducer with extracellular, transmembrane, and intracellular domains, and is overexpressed in many epithelial cancers including TNBC. It stimulates cancer cell growth and it is implicated in various metabolic pathways. TROP-2 has also been found in stem cells of various tissues, particularly in basal cells[200]. **Sacituzumab govitecan**, a humanised mAb that targets TROP2, has shown a PFS and OS benefit in mTNBC[201]. Trials are upcoming in the neoadjuvant setting (NeoSTAR, NCT04230109), and recruiting in the adjuvant setting (GBG102-SASCIA NCT04595565 as monotherapy and ASPRIA NCT04434040 in combination with immunotherapy) for patients with residual invasive disease after NACT.

Dysregulation of the *NOTCH* pathway leads to aberrant self-renewal and transformation of mammary cancer stem cells resulting in tumourigenesis[202]. Inhibition of *NOTCH* signalling has been considered an attractive strategy for the treatment of TNBC given its role in promoting EMT and cancer stem cell maintenance[203]. Preclinical and clinical studies involving γ-secretase inhibitors and mAbs against *NOTCH* receptors have explored its potential utility with encouraging results but toxicity has been limiting[204]. The subgroup of TNBC achieving the best response to the targeting of this pathway remains undefined.

Activation of *RAS/MAPK* signalling is more frequent in TNBCs compared to other BC subtypes[205]. Although canonical aberrations in the RAS, RAF, MEK or ERK genes are not found frequently in TNBC, amplification or mutations in these genes are described in approximately 6% of BC overall[18,206]. Other mechanisms for RAS/MAK activation have also been described[319]. MEK inhibitors have been trialled in unselected mTNBC with modest results[207,208]. Trials are underway in the locally advanced setting that select for hyperactivation of ERK (NCT04494958) and RAS pathway mutations (NCT05111561).

Dysregulation of the *JAK/STAT3*, *cyclinD–CDK4/6–INK4–Rb–E2F*, *TGF-β* and *WNT/B-catenin* pathways appears to be critical in TNBC development and progression. Clinical testing of the inhibition of these pathways in TNBC is still immature.

Table S5 summarises ongoing trials that target the above pathways in early TNBC.

Summary Box 8 - Other pathways: treatment strategies

- **Targeted therapies should be directed with a biomarker to best determine efficacy in the TNBC population most likely to derive benefit.**
- **Dysregulation of the *PI3K/AKT/mTOR* pathway is often observed in TNBC. Efforts to target this pathway have inconsistently shown a modest benefit.**
- **Targeting AR has shown some clinical benefit and several trials are ongoing to further evaluate this. A standardised method to determine AR pathway activation is lacking.**
- **Overall benefit of targeting the *EGFR, VEGF* and *FGFR* pathways remains modest. Lack of predictive biomarkers that identify sensitive patients has limited the clinical utility of these drugs.**
- **Treatment directed towards HER2-low TNBC has provided new therapeutic opportunities in a proportion of patients with encouraging results from trials to date.**
- **Sacituzumab govitecan, a humanised mAb that targets TROP2, has shown a PFS and OS benefit in mTNBC. It remains to be seen if this success can be translated into the early TNBC setting.**

Discussion

Improved understanding of tumour genomics, transcriptomics, epigenetics, and their interaction with the tumour microenvironment has allowed a greater insight into the true diversity of TNBCs. In addition, numerous advances in both preclinical and clinical research have directed the treatment of TNBC toward a more personalised approach. Despite the introduction of an increasing number of novel strategies in the clinical setting, approximately one third of patients diagnosed with early stage disease will have limited response to primary treatment and face a poor long term outcome. The underlying complexity of TNBC and the challenges in translating experimental science into the clinic could explain why current management approaches remain insufficient. The current therapeutic landscape for early TNBC is severely limited when compared to the large number of compounds in development. **Figure 1** shows the spectrum of agents with known or potential activity in TNBC. Only a small proportion of these reach patient care, and the pace at which these agents enter the early BC setting remains frustratingly slow. Immunotherapy and DDR agents lead the field with encouraging results.

Predictive biomarkers are not routinely used in the clinical management of early sporadic TNBC. The use of *gBRCA* mutations to select patients who could benefit from platinum-based chemotherapy and PARPi demonstrates how a molecular alteration can aid patient selection for treatment. As yet there is no definitive evidence to either support or refute the use of PARPi in the non-g*BRCA* TNBC population. An ongoing neoadjuvant study (NCT03150576) that includes both sporadic TNBC and *BRCA*-associated tumours will help to elucidate the value of *gBRCA* mutations in predicting response to the addition of PARPi to platinum-based chemotherapy[102]. Furthermore, no biomarker predicted for benefit from Pembrolizumab in the KEYNOTE-522 trial, despite the encouraging response rates shown. The expected role of PD-L1 as a biomarker of response is not proven in the early setting[138,143]. Substantial differences between the clonal architecture and the microenvironment of primary and metastatic tumours[210,211] suggest that the role of a given biomarker should be evaluated separately in both early and advanced settings.

A single biomarker strategy is unlikely to be successful for such a heterogeneous disease considering the large number of treatment strategies already tested and the increasing evidence of molecular complexity in TBNC. **Figure 2** illustrates the variety of molecular components currently explored as potential biomarkers of response and resistance. Several interactions across components also contribute to the challenge. As an example, to adequately characterise the relationship between host immunity and tumour, a single determination of the extent of immune activation is expected to be insufficient. Understanding how the immune response modulates the intrinsic genomic architecture of the tumour, and the spatial and cellular

distribution of immune cells in response to treatment appears to be crucial. Similarly, multiple pathway signalling, a common finding in TNBC tumours, could result in the activation of compensatory feedback loops that explain some mechanisms of tumour evasion and resistance when a single pathway inhibition is applied[212]. An integrative approach including tumour architecture, microenvironment, and pathway activation is more likely to succeed. A pragmatic example of how an immune-molecular profile directed approach could be implemented is shown in **Figure 3**. Tumours could be classed as 'hot' (high immune activation) or 'cold' (low immune activation) as well as 'high-burden' (high mutational/clonal burden) or 'low-burden' (low mutational/clonal burden). Hot-high burden tumours are frequently highly proliferative and more likely to exhibit high chromosomal instability. Increased response to cytotoxic and immunotherapy agents is anticipated in this subgroup. The hot- low burden group represent a subgroup in which clonal selection has been enforced by an active immune system. This good prognosis subgroup is likely to require less intensive therapies with treatments focused on targeting key drivers. In sharp contrast, cold tumours, require more comprehensive approaches that often include treatment escalation strategies. It is possible that due to quiescent mechanisms of tumorigenesis cold tumours remain invisible to the immune system. Therefore, sequential strategies that aim to enhance the immune system effect are essential in this group. In cold-low burden tumours, targeted pathway inactivation followed by immune checkpoint inhibition could potentially result in an augmented immune response achieving long-lasting control of the disease. Cold-high burden tumours constitute a poor prognosis group with patent mechanisms of immune evasion. Sequential strategies that include immunotherapy followed by either chemotherapy, pathway-specific targeted agents, radiotherapy-targeted agent combinations are plausible options.

Response to NACT, measured as the amount of residual disease found at surgery, has recently been used as a primary endpoint to test novel agents in the early setting. RCB is widely considered a prognostic factor and is frequently used as a surrogate endpoint for long-term outcomes, particularly in this BC subgroup. Although it is clear from a recent meta-analyses that RCB is a better endpoint than pCR, the identification of the molecular characteristics that explain why some tumours do not follow the predicted outcomes (recurrences after excellent responses or long-lasting EFS after residual disease) continues to present a challenge. There is robust evidence that supports the association between RCB score and long-term outcome in patients that have received NACT[79]. Evidence for the predictive value of RCB in the context of targeted therapy is lacking and requires further investigation[167,168]. Multiple other methodologies to aid the identification of patients with higher disease relapse risk are currently being explored. The post neoadjuvant and adjuvant settings are an excellent opportunity to evaluate the contribution of dynamic biomarkers (e.g RCB, TILs) to enable an accurate selection of patients that may benefit from escalating treatment strategies. Pre- and post-treatment assessment of ctDNA and TME plus integration of traditional transcriptomic and genomic signatures or classifications are some of the more promising approaches. Alternatively, innovative adaptive trial designs that enable early response assessment and facilitate an early change in management could minimise overtreatment and appropriately de-escalate or escalate therapy when appropriate.

Several molecular predictors of response that incorporate a variety of 'omic' data to aid clinical decisions have been developed. Limited clinical impact has been derived due to a lack of reproducibility, lengthy timeline of results, and expense. The real-time delivery of genomic and transcriptomic results will facilitate the implementation of adaptive trial designs and permit the investigation of novel and existing biomarkers. There are multiple pan-cancer studies assessing the implementation of genomics and transcriptomics into clinical care, for example, the UK 100,000 Genomes Project[213], the Dutch national Centre for Personalised Cancer Treatment (CPCT) study[214], and the Personalised Onco-Genomics (POG) Program[215]. The Personalised Breast Cancer Programme (PBCP)[216] is a tumour-specific precision medicine project that implements whole-genome sequencing data into the real-time treatment of early and advanced breast cancer patients. This programme ensures the delivery of high quality annotated genomic data to patients and clinicians while promoting hypothesis testing and tumour-specific analysis. It is clear that these large-scale sequencing studies will add considerably to our understanding and enable better optimisation of trial design, response prediction, prognostication, and biomarker discovery. These efforts, combined with

the promising potential of novel agents and treatment combinations, gives us the exciting prospect of a tailored treatment pathway for each patient diagnosed with early-stage TNBC.

The ultimate aim is that every patient diagnosed with early-stage TNBC has a bespoke treatment pathway developed that fits their TNBC. The individualised use of preclinical models such as patient-derived organoids or xenografts[216], and the implementation of advanced radiodiagnostic techniques[217] are pivotal to achieving this goal. This type of integrated approach requires open and clear communication and collaboration between basic scientists, clinicians, and other scientific disciplines, for example, bioinformatics, mathematics, and physics, which will maximise the chance of success and ultimately enhance patient benefit.

In conclusion, advances in tumour characterisation, real-time biomarker/genomic testing, trial design and drug development provide the foundation for an era of precision therapeutic strategies in early TNBC. In the development of complex strategies to integrate multi-modal data to derive individualised care plans, it is important to consider the holistic needs of each patient to achieve a truly personalised approach.

Acronyms

AR	Androgen receptor
BC	Breast cancer
BL1	Basal-like 1
BL2	Basal-like 2
BLIA	Basal-like immune activated
BLIS	Basal-like immunosuppressed
ctDNA	Circulating tumour DNA
DDR	DNA damage response
DFS	Disease free survival
DSBs	Double strand breaks
EBCTCG	Early Breast Cancer Trailist's Collaborative Group
EFS	Event free survival
EMT	Epithelial-mesenchymal transition
ER	Oestrogen receptor
g*BRCA*	Germline *BRCA*
GE	Gene expression
HR	Homologous recombination
HRD	Homologous recombination deficiency
ICI	Immune checkpoint inhibitors
iDFS	Invasive disease free survival
IntClust	Integrative Cluster
iTILs	Intratumoural TILs
LAR	Luminal androgen receptor
M	Mesenchymal -Lehmann subtype
mAbs	Monoclonal antibodies
mTNBC	Metastatic triple negative breast cancer
NACT	Neoadjuvant chemotherapy
OR	Overall response
OS	Overall survival
PARP	Poly ADP-ribose polymerase
PARPi	Poly ADP-ribose polymerase inhibitors
pCR	Pathological complete response
PD-1	Programmed cell death 1
PD-L1	Programmed cell death ligand-1
PFS	Progression free survival
PR	Progesterone receptor
RCB	Residual Cancer Burden

RFS	Relapse-free survival
ssDNA	Single strand DNA
sTILs	Stromal TILs
TILs	Tumour infiltrating lymphocytes
TMB	Tumour mutational burden
TNBC	Triple negative breast cancer

Tables

Provided in a separate file.

References

1. Wild CP, W. E. S. B. editors (2020). World Cancer Report: Cancer Research for Cancer Prevention. Lyon, France.

2. Penault-Llorca, F., and Viale, G. (2012). Pathological and molecular diagnosis of triple-negative breast cancer: a clinical perspective. Annals of Oncology 23. doi:10.1093/annonc/mds190.

3. Bauer, K. R., Brown, M., Cress, R. D., Parise, C. A., and Caggiano, V. (2007). Descriptive analysis of oestrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype. Cancer 109. doi:10.1002/cncr.22618.

4. Steward, L., Conant, L., Gao, F., and Margenthaler, J. A. (2014). Predictive Factors and Patterns of Recurrence in Patients with Triple Negative Breast Cancer. Annals of Surgical Oncology 21. doi:10.1245/s10434-014-3546-4.

5. Dent, R., Trudeau, M., Pritchard, K. I., Hanna, W. M., Kahn, H. K., Sawka, C. A., et al. (2007). Triple-Negative Breast Cancer: Clinical Features and Patterns of Recurrence. Clinical Cancer Research 13. doi:10.1158/1078-0432.CCR-06-3045.

6. Pereira, B., Chin, S.-F., Rueda, O. M., Vollan, H.-K. M., Provenzano, E., Bardwell, H. A., et al. (2016). The somatic mutation profiles of 2,433 breast cancers refine their genomic and transcriptomic landscapes. Nature Communications7, 11479. doi:10.1038/ncomms11479.

7. Shah, S. P., Roth, A., Goya, R., Oloumi, A., Ha, G., Zhao, Y., et al. (2012a). The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 486, 395–399. doi:10.1038/nature10933

8. Bianchini, G., Balko, J. M., Mayer, I. A., Sanders, M. E., and Gianni, L. (2016). Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nature Reviews Clinical Oncology 13, 674–690. doi:10.1038/nrclinonc.2016.66.

9. Balkenhol, M. C. A., Vreuls, W., Wauters, C. A. P., Mol, S. J. J., van der Laak, J. A. W. M., & Bult, P. (2020). Histological subtypes in triple negative breast cancer are associated with specific information on survival. Annals of Diagnostic Pathology, 46, 151490. https://doi.org/10.1016/J.AN§NDIAGPATH.2020.151490

10. Pareja, F., Geyer, F. C., Marchiò, C., Burke, K. A., Weigelt, B., and Reis-Filho, J. S. (2016). Triple-negative breast cancer: The importance of molecular and histologic subtyping, and recognition of low-grade variants. npj Breast Cancer 2, 1–11. doi:10.1038/npjbcancer.2016.36.

11. Bergeron, A., MacGrogan, G., Bertaut, A., Ladoire, S., Arveux, P., Desmoulins, I., et al. (2021). Triple-negative breast lobular carcinoma: a luminal androgen receptor carcinoma with specific ESRRA mutations. Modern Pathology 34, 1282–1296. doi:10.1038/s41379-021-00742-9.

12. Ng, C. K. Y., Piscuoglio, S., Geyer, F. C., Burke, K. A., Pareja, F., Eberle, C. A., et al. (2017). The landscape of somatic genetic alterations in metaplastic breast carcinomas. Clinical Cancer Research 23, 3859–3870. doi:10.1158/1078-0432.CCR-16-2857.

13. Bertucci, F., Finetti, P., Cervera, N., Charafe-Jauffret, E., Mamessier, E., Adélaïde, J., et al. (2006). Gene expression profiling shows medullary breast cancer is a subgroup of basal breast cancers. Cancer Research66, 4636–4644. doi:10.1158/0008-5472.CAN-06-0031.

14. Sun, X., Zuo, K., Yao, Q., Zhou, S., Shui, R., Xu, X., et al. (2020). Invasive apocrine carcinoma of the breast: clinicopathologic features and comprehensive genomic profiling of 18 pure triple-negative apocrine carcinomas. Modern Pathology 33, 2473–2482. doi:10.1038/s41379-020-0589-x.

15. Martelotto, L. G., De Filippo, M. R., Ng, C. K., Natrajan, R., Fuhrmann, L., Cyrta, J., et al. (2015). Genomic landscape of adenoid cystic carcinoma of the breast. Journal of Pathology 237, 179–189. doi:10.1002/path.4573.

16. Tognon, C., Knezevich, S. R., Huntsman, D., Roskelley, C. D., Melnyk, N., Mathers, J. A., et al. (2002). Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. Cancer Cell 2, 367–376. doi:10.1016/S1535-6108(02)00180-0.

17. Perou, C. M., Sørlie, T., Eisen, M. B., van de Rijn, M., Jeffrey, S. S., Rees, C. A., et al. (2000). Molecular portraits of human breast tumours. Nature 406, 747–752. doi:10.1038/35021093.

18. Cancer Genome Atlas Network (2012). Comprehensive molecular portraits of human breast tumours. Nature 490, 61–70. doi:10.1038/nature11412.

19. Pommier, R. M., Sanlaville, A., Tonon, L., Kielbassa, J., Thomas, E., Ferrari, A., et al. (2020). Comprehensive characterization of claudin-low breast tumours reflects the impact of the cell-of-origin on cancer evolution. Nature Communications 11, 3431. doi:10.1038/s41467-020-17249-7

20. Prat, A., Parker, J. S., Karginova, O., Fan, C., Livasy, C., Herschkowitz, J. I., et al. (2010). Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Research12, R68. doi:10.1186/bcr2635.

21. Metzger Filho, O., Stover, D. G., Asad, S., Ansell, P. J., Watson, M., Loibl, S., et al. (2019). Immunophenotype and proliferation to predict for response to neoadjuvant chemotherapy in TNBC: Results from BrighTNess phase III study. Journal of Clinical Oncology 37, 510–510. doi:10.1200/JCO.2019.37.15_suppl.510.

22. Sikov, W. M., Barry, W. T., Hoadley, K. A., Pitcher, B. N., Singh, B., Tolaney, S. M., et al. (2015). Abstract S4-05: Impact of intrinsic subtype by PAM50 and other gene signatures on pathologic complete response (pCR) rates in triple-negative breast cancer (TNBC) after neoadjuvant chemotherapy (NACT) +/- Carboplatin (Cb) or bevacizumab (Bev): CALGB 40603. in General Session Abstracts (American Association for Cancer Research), S4-05-S4-05. doi:10.1158/1538-7445.SABCS14-S4-05.

23. Lehmann, B. D., and Pietenpol, J. A. (2014). Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. The Journal of Pathology 232, 142–150. doi:10.1002/path.4280.

24. Lehmann, B. D., Jovanović, B., Chen, X., Estrada, M. v., Johnson, K. N., Shyr, Y., et al. (2016). Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. PLOS ONE 11. doi:10.1371/journal.pone.0157368.

25. Masuda, H., Baggerly, K. A., Wang, Y., Zhang, Y., Gonzalez-Angulo, A. M., Meric-Bernstam, F., et al. (2013). Differential Response to Neoadjuvant Chemotherapy Among 7 Triple-Negative Breast Cancer Molecular Subtypes. Clinical Cancer Research 19. doi:10.1158/1078-0432.CCR-13-0799

26. Santonja, A., Sánchez-Muñoz, A., Lluch, A., Chica-Parrado, M. R., Albanell, J., Chacón, J. I., et al. (2018). Triple negative breast cancer subtypes and pathologic complete response rate to neoadjuvant chemotherapy. Oncotarget 9, 26406–26416. doi:10.18632/oncotarget.25413.

27. Burstein, M. D., Tsimelzon, A., Poage, G. M., Covington, K. R., Contreras, A., Fuqua, S. A. W., et al. (2015). Comprehensive Genomic Analysis Identifies Novel Subtypes and Targets of Triple-Negative Breast Cancer. Clinical Cancer Research 21. doi:10.1158/1078-0432.CCR-14-0432.

28. Liu, Y.-R., Jiang, Y.-Z., Xu, X.-E., Yu, K.-D., Jin, X., Hu, X., et al. (2016). Comprehensive transcriptome analysis identifies novel molecular subtypes and subtype-specific RNAs of triple-negative breast cancer. Breast Cancer Research 18. doi:10.1186/s13058-016-0690-8.

29. Jiang, Y.-Z., Ma, D., Suo, C., Shi, J., Xue, M., Hu, X., et al. (2019). Genomic and Transcriptomic Landscape of Triple-Negative Breast Cancers: Subtypes and Treatment Strategies. Cancer Cell 35, 428-440.e5. doi:10.1016/j.ccell.2019.02.001.

30. Curtis, C., Shah, S. P., Chin, S.-F. F., Turashvili, G., Rueda, O. M., Dunning, M. J., et al. (2012). The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 486, 346–352. doi:10.1038/nature10983.

31. Rueda, O. M., Sammut, S.-J., Seoane, J. A., Chin, S.-F., Caswell-Jin, J. L., Callari, M., et al. (2019). Dynamics of breast-cancer relapse reveal late-recurring ER-positive genomic subgroups. Nature 567, 399–404. doi:10.1038/s41586-019-1007-8.

32. Telli, M. L., Timms, K. M., Reid, J., Hennessy, B., Mills, G. B., Jensen, K. C., et al. (2016). Homologous. Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant Chemotherapy in Patients with Triple-Negative Breast Cancer. Clinical Cancer Research 22, 3764–3773. doi:10.1158/1078-0432.CCR-15-2477.

33. Prado-Vázquez, G., Gámez-Pozo, A., Trilla-Fuertes, L., Arevalillo, J. M., Zapater-Moros, A., Ferrer-Gómez, M., et al. (2019). A novel approach to triple-negative breast cancer molecular classification reveals a luminal immune-positive subgroup with good prognoses. Scientific Reports 9, 1538. doi:10.1038/s41598-018-38364-y.

34. Schmid, P., Cortes, J., Dent, R., Pusztai, L., McArthur, H., Kümmel, S., et al. (2022). Event-free Survival with Pembrolizumab in Early Triple-Negative Breast Cancer. New England Journal of Medicine 386, 556–567. doi:10.1056/NEJMOA2112651/SUPPL_FILE/NEJMOA2112651_DATA-SHARING.PDF.

35. Tutt, A. N. J., Garber, J. E., Kaufman, B., Viale, G., Fumagalli, D., Rastogi, P., Gelber, R. D., de Azambuja, E., Fielding, A., Balmaña, J., Domchek, S. M., Gelmon, K. A., Hollingsworth, S. J., Korde, L. A., Linderholm, B., Bandos, H., Senkus, E., Suga, J. M., Shao, Z., … Geyer, C. E. (2021). Adjuvant Olaparib for Patients with *BRCA*1 - or *BRCA*2 -Mutated Breast Cancer . New England Journal of Medicine, 384(25), 2394–2405. doi:10.1056/nejmoa2105215

36. Early Breast Cancer Trialists' Collaborative Group (EBCTCG) (2012). Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100 000 women in 123 randomised trials. The Lancet 379. doi:10.1016/S0140-6736(11)61625-5.

37. Blum, J. L., Flynn, P. J., Yothers, G., Asmar, L., Geyer, C. E., Jacobs, S. A., et al. (2017). Anthracyclines in Early Breast Cancer: The ABC Trials—USOR 06-090, NSABP B-46-I/USOR 07132, and NSABP B-49 (NRG Oncology). Journal of Clinical Oncology 35. doi:10.1200/JCO.2016.71.4147.

38. Braybrooke, J., Bradley, R., Gray, R., Hills R., Liu Z., Pan H., Peto R., Blum J, Chen X., Ejlertsen B., Janni W., Nitz U., Slamon D., Toi M., Watanabe T., Swain S.,Bergh J. (2021). Abstract GS2-06. Taxane with anthracycline versus taxane without anthracycline: An individual patient-level meta-analysis of 16,500 women with early-stage breast cancer in 13 randomised trials. https://www.abstractsonline.com/pp8/#!/10462/presentation/646 [Accessed March 14, 2022]

39. John, P., Osani, M. C., Kodali, A., Buchsbaum, R., Bannuru, R. R., & Erban, J. K. (2021). Comparative Effectiveness of Adjuvant Chemotherapy in Early-Stage Breast Cancer: A Network Meta-analysis. Clinical breast cancer, 21(1), e22–e37. doi:10.1200/JCO.2017.35.15_suppl.e12071 Journal of Clinical Oncology 35, no. 15_suppl

40. Hurvitz, S. A., McAndrew, N. P., Bardia, A., Press, M. F., Pegram, M., Crown, J. P., Fasching, P. A., Ejlertsen, B., Yang, E. H., Glaspy, J. A., & Slamon, D. J. (2021). A careful reassessment of anthracycline use in curable breast cancer. Npj Breast Cancer 2021 7:1, 7(1), 1–25. https://doi.org/10.1038/s41523-021-00342-5

41. Yu, K.-D., Liu, X.-Y., Chen, L., Mo, M., Wu, J., Liu, G.-Y., et al. (2021). Anthracycline-free or short-term regimen as adjuvant chemotherapy for operable breast cancer: A phase III randomised non-inferiority trial. The Lancet Regional Health - Western Pacific 11, 100158. doi:10.1016/j.lanwpc.2021.100158.

42. Ejlertsen, B., Tuxen, M. K., Jakobsen, E. H., Jensen, M.-B., Knoop, A. S., Højris, I., et al. (2017). Adjuvant Cyclophosphamide and Docetaxel With or Without Epirubicin for Early TOP2A -Normal Breast Cancer: DBCG

07-READ, an Open-Label, Phase III, randomised Trial. Journal of Clinical Oncology 35, 2639–2646. doi:10.1200/JCO.2017.72.3494.

43. Mavroudis, D., Matikas, A., Malamos, N., Papakotoulas, P., Kakolyris, S., Boukovinas, I., et al. (2016). Dose-dense FEC followed by docetaxel versus docetaxel plus cyclophosphamide as adjuvant chemotherapy in women with HER2-negative, axillary lymph node-positive early breast cancer: a multicenter randomised study by the Hellenic Oncology Research Group (HORG). Annals of Oncology27, 1873–1878. doi:10.1093/annonc/mdw274.

44. Janni, W., Nitz, U., Rack, B. K., Gluz, O., Schneeweiss, A., Kates, R. E., et al. (2018). Pooled analysis of two randomised phase III trials (PlanB/SuccessC) comparing six cycles of docetaxel and cyclophosphamide to sequential anthracycline taxane chemotherapy in patients with intermediate and high risk HER2-negative early breast cancer (n=5,923). Journal of Clinical Oncology 36, 522–522. doi:10.1200/JCO.2018.36.15_suppl.522.

45. Gianni L, Baselga J, Eiermann W, Guillem Porta V, Semiglazov V, et al. European Cooperative Trial in Operable Breast Cancer (ECTO): Improved freedom from progression (FFP) from adding paclitaxel (T) to doxorubicin (A) followed by cyclophosphamide methotrexate and fluorouracil (CMF). J Clin Oncol ASCO. 2005;(suppl 16):abstr 513

46. Untch, M., Jackisch, C., Schneeweiss, A., Conrad, B., Aktas, B., Denkert, C., et al. (2016). Nab-Paclitaxel versus solvent-based Paclitaxel in neoadjuvant chemotherapy for early breast cancer (GeparSepto—GBG 69): a randomised, phase 3 trial. The Lancet Oncology 17, 345–356. doi:10.1016/S1470-2045(15)00542-2.

47. Gianni, L., Mansutti, M., Anton, A., Calvo, L., Bisagni, G., Bermejo, B., et al. (2018). Comparing Neoadjuvant Nab-Paclitaxel vs Paclitaxel Both Followed by Anthracycline Regimens in Women With ERBB2/HER2 -Negative Breast Cancer—The Evaluating Treatment With Neoadjuvant Abraxane (ETNA) Trial. JAMA Oncology 4, 302. doi:10.1001/jamaoncol.2017.4612.

48. Gluz, O., Nitz, U., Liedtke, C., Christgen, M., Grischke, E.-M., Forstbauer, H., et al. (2018). Comparison of Neoadjuvant Nab-Paclitaxel+Carboplatin vs Nab-Paclitaxel+gemcitabine in Triple-Negative Breast Cancer: randomised WSG-ADAPT-TN Trial Results. JNCI: Journal of the National Cancer Institute 110, 628–637. doi:10.1093/jnci/djx258.

49. Mittendorf, E. A., Zhang, H., Barrios, C. H., Saji, S., Jung, K. H., Hegg, R., et al. (2020). Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. The Lancet 396, 1090–1100. doi:10.1016/S0140-6736(20)31953-X.

50. Yardley, D. A., Arrowsmith, E. R., Daniel, B. R., Eakle, J., Brufsky, A., Drosick, D. R., et al. (2017). TITAN: phase III study of doxorubicin/cyclophosphamide followed by ixabepilone or paclitaxel in early-stage triple-negative breast cancer. Breast Cancer Research and Treatment 164, 649–658. doi:10.1007/s10549-017-4285-6.

51. Poggio F, Bruzzone M, Ceppi M, et al. Platinum-based neoadjuvant chemotherapy in triple-negative breast cancer: a systematic review and meta-analysis. Ann Oncol. doi: 10.1093/annonc/mdy127.

52. Li J, Yu K, Pang D, et al. Adjuvant Capecitabine With Docetaxel and Cyclophosphamide Plus Epirubicin for Triple-Negative Breast Cancer (CBCSG010): An Open-Label, Randomized, Multicenter, Phase III Trial. J Clin Oncol. 2020;38(16):1774-1784. doi:10.1200/JCO.19.02474

53. Joensuu H, Kellokumpu-Lehtinen P, Huovinen R, et al. Adjuvant Capecitabine in Combination With Docetaxel, Epirubicin, and Cyclophosphamide for Early Breast Cancer: The Randomized Clinical FinXX Trial. JAMA Oncol. 2017;3(6):793–800. doi:10.1001/jamaoncol.2016.6120

54. Ye, F., Bian, L., Wen, J. et al. Additional capecitabine use in early-stage triple negative breast cancer patients receiving standard chemotherapy: a new era? A meta-analysis of randomized controlled trials. BMC Cancer 22, 261 (2022). https://doi.org/10.1186/s12885-022-09326-5

55. Early Breast Cancer Trialists' Collaborative Group (2015). Adjuvant bisphosphonate treatment in early breast cancer: meta-analyses of individual patient data from randomised trials. The Lancet386, 1353–1361. doi:10.1016/S0140-6736(15)60908-4.

56. Coleman, R. E., Collinson, M., Gregory, W., Marshall, H., Bell, R., Dodwell, D., et al. (2018). Benefits and risks of adjuvant treatment with zoledronic acid in stage II/III breast cancer. 10 years follow-up of the AZURE randomised clinical trial (BIG 01/04). Journal of Bone Oncology 13, 123–135. doi:10.1016/j.jbo.2018.09.008.

57. von Minckwitz, G., Rezai, M., Tesch, H., Huober, J., Gerber, B., Zahm, D. M., et al. (2016). Zoledronate for patients with invasive residual disease after anthracyclines-taxane-based chemotherapy for early breast cancer – The Phase III NeoAdjuvant Trial Add-oN (NaTaN) study (GBG 36/ABCSG 29). European Journal of Cancer 64, 12–21. doi:10.1016/J.EJCA.2016.05.015.

58. Coleman, R., Winter, M., Cameron, D. et al. The effects of adding zoledronic acid to neoadjuvant chemotherapy on tumour response: exploratory evidence for direct anti-tumour activity in breast cancer. Br J Cancer 102, 1099–1105 (2010). https://doi.org/10.1038/sj.bjc.6605604

59. Coleman, R., Finkelstein, D. M., Barrios, C., Martin, M., Iwata, H., Hegg, R., et al. (2020). Adjuvant denosumab in early breast cancer (D-CARE): an international, multicentre, randomised, controlled, phase 3 trial. The Lancet Oncology 21. doi:10.1016/S1470-2045(19)30687-4.

60. Wolmark, N., Wang, J., Mamounas, E., Bryant, J., and Fisher, B. (2001). Preoperative Chemotherapy in Patients With Operable Breast Cancer: Nine-Year Results From National Surgical Adjuvant Breast and Bowel Project B-18. JNCI Monographs 2001. doi:10.1093/oxfordjournals.jncimonographs.a003469.

61. van der Hage, J. A., van de Velde, C. J. H., Julien, J.-P., Tubiana-Hulin, M., Vandervelden, C., and Duchateau, L. (2001). Preoperative Chemotherapy in Primary Operable Breast Cancer: Results From the European Organization for Research and Treatment of Cancer Trial 10902. Journal of Clinical Oncology 19. doi:10.1200/JCO.2001.19.22.4224.

62. Mauriac, L., MacGrogan, G., Avril, A., Durand, M., Floquet, A., Debled, M., et al. (1999). Neoadjuvant chemotherapy for operable breast carcinoma larger than 3 cm: A unicentre randomised trial with a 124-month median follow-up. Annals of Oncology 10. doi:10.1023/A:1008337009350.

63. Early Breast Cancer Trialists' Collaborative Group (EBCTCG) (2018). Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: meta-analysis of individual patient data from ten randomised trials. The Lancet. Oncology 19, 27–39. doi:10.1016/S1470-2045(17)30777-5.

64. Karagiannis GS, Pastoriza JM, Wang Y, et al. Neoadjuvant chemotherapy induces breast cancer metastasis through a TMEM-mediated mechanism [published correction appears in Sci Transl Med. 2017 Jul 19;9(399):]. Sci Transl Med. 2017;9(397):eaan0026. doi:10.1126/scitranslmed.aan0026

65. Xia, L.-Y., Hu, Q.-L., Zhang, J., Xu, W.-Y., and Li, X.-S. (2020). Survival outcomes of neoadjuvant versus adjuvant chemotherapy in triple-negative breast cancer: a meta-analysis of 36,480 cases. World Journal of Surgical Oncology 18. doi:10.1186/s12957-020-01907-7.

66. Symmans, W. F., Wei, C., Gould, R., Yu, X., Zhang, Y., Liu, M., et al. (2017). Long-Term Prognostic Risk After Neoadjuvant Chemotherapy Associated With Residual Cancer Burden and Breast Cancer Subtype. Journal of Clinical Oncology 35. doi:10.1200/JCO.2015.63.1010.

67. Thompson, A. M., and Moulder-Thompson, S. L. (2012). Neoadjuvant treatment of breast cancer. Annals of Oncology 23. doi:10.1093/annonc/mds324.

68. Shao, N., Wang, S., Yao, C., Xu, X., Zhang, Y., Zhang, Y., et al. (2012). Sequential versus concurrent anthracyclines and taxanes as adjuvant chemotherapy of early breast cancer: A meta-analysis of phase III randomised control trials. The Breast 21. doi:10.1016/j.breast.2012.03.011.

69. Earl, H. M., Vallier, A.-L., Hiller, L., Fenwick, N., Young, J., Iddawela, M., et al. (2014). Effects of the addition of gemcitabine, and Paclitaxel-first sequencing, in neoadjuvant sequential epirubicin, Cyclophosphamide, and Paclitaxel for women with high-risk early breast cancer (Neo-tAnGo): an open-label, 2×2 factorial randomised phase 3 trial. The Lancet Oncology 15, 201–212. doi:10.1016/S1470-2045(13)70554-0.

70. Amir, E., Ocana, A., Freedman, O., Clemons, M., and Seruga, B. (2010). Dose-dense treatment for triple-negative breast cancer. Nature Reviews Clinical Oncology 7. doi:10.1038/nrclinonc.2009.23

71. Bonilla, L., Ben-Aharon, I., Vidal, L., Gafter-Gvili, A., Leibovici, L., and Stemmer, S. M. (2010). Dose-Dense Chemotherapy in Nonmetastatic Breast Cancer: A Systematic Review and Meta-analysis of randomised Controlled Trials. JNCI Journal of the National Cancer Institute 102. doi:10.1093/jnci/djq409.

72. Ding, Y., Ding, K., Yang, H., He, X., Mo, W., and Ding, X. (2020). Does dose-dense neoadjuvant chemotherapy have clinically significant prognostic value in breast cancer?: A meta-analysis of 3,724 patients. PLOS ONE 15. doi:10.1371/journal.pone.0234058.

73. Gray, R., Bradley, R., Braybrooke, J., Liu, Z., Peto, R., Davies, L., et al. (2019). Increasing the dose intensity of chemotherapy by more frequent administration or sequential scheduling: a patient-level meta-analysis of 37 298 women with early breast cancer in 26 randomised trials. The Lancet 393, 1440–1452. doi:10.1016/S0140-6736(18)33137-4/ATTACHMENT/4A5A797F-D8DD-4E81-B17F-E8F9CE7116FF/MMC1.PDF.

74. Hanahan, D., Bergers, G., & Bergsland, E. (2000). Less is more, regularly: metronomic dosing of cytotoxic drugs can target tumour angiogenesis in mice. Journal of Clinical Investigation, 105(8), 1045. https://doi.org/10.1172/JCI9872

75. Wang, X., Wang, S.-S., Huang, H., Cai, L., Zhao, L., Peng, R.-J., Lin, Y., Tang, J., Zeng, J., Zhang, L.-H., Ke, Y.-L., Wang, X.-M., Liu, X.-M., Chen, Q.-J., Zhang, A.-Q., Xu, F., Bi, X.-W., Huang, J.-J., Li, J.-B., … Yuan, Z.-Y. (2021). Effect of Capecitabine Maintenance Therapy Using Lower Dosage and Higher Frequency vs Observation on Disease-Free Survival Among Patients With Early-Stage Triple-Negative Breast Cancer Who Had Received Standard Treatment. JAMA, 325(1), 50. https://doi.org/10.1001/jama.2020.23370

76. Colleoni, M., Gray, K. P., Gelber, S., Láng, I., Thürlimann, B., Gianni, L., et al. (2016). Low-Dose Oral Cyclophosphamide and Methotrexate Maintenance for Hormone Receptor–Negative Early Breast Cancer: International Breast Cancer Study Group Trial 22-00. Journal of Clinical Oncology 34, 3400–3408. doi:10.1200/JCO.2015.65.6595.

77. Huang, M., O'Shaughnessy, J., Zhao, J., Haiderali, A., Cortés, J., Ramsey, S. D., et al. (2020). Association of Pathologic Complete Response with Long-Term Survival Outcomes in Triple-Negative Breast Cancer: A Meta-Analysis. Cancer Research 80, 5427–5434. doi:10.1158/0008-5472.CAN-20-1792.

78. Carey, L. A., Dees, E. C., Sawyer, L., Gatti, L., Moore, D. T., Collichio, F., et al. (2007). The Triple Negative Paradox: Primary tumour Chemosensitivity of Breast Cancer Subtypes. Clinical Cancer Research 13. doi:10.1158/1078-0432.CCR-06-1109.

79. Yau, C., Osdoit, M., Noordaa, M. van der, Shad, S., Wei, J., Croze, D. de, Hamy, A.-S., Laé, M., Reyal, F., Sonke, G. S., Steenbruggen, T. G., Seijen, M. van, Wesseling, J., Martín, M., Monte-Millán, M. del, López-Tarruella, S., Adamson, K., Albain, K. S., Asare, A. L., … Symmans, W. F. (2022). Residual cancer burden after neoadjuvant chemotherapy and long-term survival outcomes in breast cancer: a multicentre pooled analysis of 5161 patients. The Lancet Oncology, 23(1), 149–160. https://doi.org/10.1016/S1470-2045(21)00589-1

80. Cavallone, L., Aguilar-Mahecha, A., Lafleur, J., Brousse, S., Aldamry, M., Roseshter, T., et al. (2020). Prognostic and predictive value of circulating tumour DNA during neoadjuvant chemotherapy for triple negative breast cancer. Scientific Reports 10, 14704. doi:10.1038/s41598-020-71236-y.

81. Magbanua, M. J. M., Swigart, L. B., Wu, H. T., Hirst, G. L., Yau, C., Wolf, D. M., et al. (2021). Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Annals of Oncology 32, 229–239.

doi:10.1016/J.ANNONC.2020.11.007/ATTACHMENT/E73EC62B-DC11-49A9-942F-DBA96CD2C123/MMC1.DOCX.

82. Masuda, N., Lee, S.-J., Ohtani, S., Im, Y.-H., Lee, E.-S., Yokota, I., Kuroi, K., Im, S.-A., Park, B.-W., Kim, S.-B., Yanagita, Y., Ohno, S., Takao, S., Aogi, K., Iwata, H., Jeong, J., Kim, A., Park, K.-H., Sasano, H., … Toi, M. (2017). Adjuvant Capecitabine for Breast Cancer after Preoperative Chemotherapy. New England Journal of Medicine, 376(22). https://doi.org/10.1056/NEJMoa1612645

83. Lluch, A., Barrios, C. H., Torrecillas, L., Ruiz-Borrego, M., Bines, J., Segalla, J., Guerrero-Zotano, Á., García-Sáenz, J. A., Torres, R., de la Haba, J., García-Martínez, E., Gómez, H. L., Llombart, A., Bofill, J. S., Baena-Cañada, J. M., Barnadas, A., Calvo, L., Pérez-Michel, L., Ramos, M., … Martín, M. (2020). Phase III Trial of Adjuvant Capecitabine After Standard Neo-/Adjuvant Chemotherapy in Patients With Early Triple-Negative Breast Cancer (GEICAM/2003-11_CIBOMA/2004-01). Journal of Clinical Oncology, 38(3), 203–213. https://doi.org/10.1200/JCO.19.00904

84. Huo, X., Li, J., Zhao, F., Ren, D., Ahmad, R., Yuan, X., Du, F., & Zhao, J. (2021). The role of capecitabine-based neoadjuvant and adjuvant chemotherapy in early-stage triple-negative breast cancer: a systematic review and meta-analysis. BMC cancer, 21(1), 78. https://doi.org/10.1186/s12885-021-07791-y

85. Burstein, H. J., Curigliano, G., Thürlimann, B., Weber, W. P., Poortmans, P., Regan, M. M., et al. (2021). Customizing local and systemic therapies for women with early breast cancer: the St. Gallen International Consensus Guidelines for treatment of early breast cancer 2021. doi:10.1016/j.annonc.2021.06.023.

86. Beyerlin, K., Jimenez, R., Zangardi, M., Fell, G. G., Edmonds, C., Johnson, A., Bossuyt, V., Specht, M., Mulvey, T. M., Moy, B., Ellisen, L. W., Isakoff, S. J., Bardia, A., & Spring, L. M. (2020). The adjuvant use of capecitabine for residual disease following pre-operative chemotherapy for breast cancer: Challenges applying CREATE-X to a US population. Journal of Oncology Pharmacy Practice. https://doi.org/10.1177/1078155220971751

87. Schneider, B. P., Jiang, G., Ballinger, T. J., Shen, F., Chitambar, C., Nanda, R., et al. (2022). BRE12-158: A Postneoadjuvant, Randomized Phase II Trial of Personalized Therapy Versus Treatment of Physician's Choice for Patients With Residual Triple-Negative Breast Cancer. Journal of Clinical Oncology 40, 345–355. doi:10.1200/JCO.21.01657.

88. Birkbak, N. J., Eklund, A. C., Li, Q., McClelland, S. E., Endesfelder, D., Tan, P., Tan, I. B., Richardson, A. L., Szallasi, Z., & Swanton, C. (2011). Paradoxical Relationship between Chromosomal Instability and Survival Outcome in Cancer. Cancer Research, 71(10), 3447–3452. https://doi.org/10.1158/0008-5472.CAN-10-3667

89. den Brok, W. D., Schrader, K. A., Sun, S., Tinker, A. v., Zhao, E. Y., Aparicio, S., & Gelmon, K. A. (2017). Homologous Recombination Deficiency in Breast Cancer: A Clinical Review. JCO Precision Oncology, 1, 1–13. https://doi.org/10.1200/PO.16.00031

90. Evans, D. G., Howell, A., Ward, D., Lalloo, F., Jones, J. L., & Eccles, D. M. (2011). Prevalence of *BRCA*1 and *BRCA*2 mutations in triple negative breast cancer. Journal of Medical Genetics, 48(8). https://doi.org/10.1136/jmedgenet-2011-100006

91. Davies, H., Glodzik, D., Morganella, S., Yates, L. R., Staaf, J., Zou, X., Ramakrishna, M., Martin, S., Boyault, S., Sieuwerts, A. M., Simpson, P. T., King, T. A., Raine, K., Eyfjord, J. E., Kong, G., Borg, Å., Birney, E., Stunnenberg, H. G., van de Vijver, M. J., … Nik-Zainal, S. (2017). HRDetect is a predictor of *BRCA*1 and *BRCA*2 deficiency based on mutational signatures. Nature Medicine, 23(4), 517–525. https://doi.org/10.1038/nm.4292

92. Chen, X. S., Nie, X. Q., Chen, C. M., Wu, J. Y., Wu, J., Lu, J. S., Shao, Z. M., Shen, Z. Z., & Shen, K. W. (2010). Weekly paclitaxel plus carboplatin is an effective nonanthracycline-containing regimen as neoadjuvant chemotherapy for breast cancer. Annals of Oncology, 21(5), 961–967. https://doi.org/10.1093/annonc/mdq041

93. Kern, P., Kalisch, A., von Minckwitz, G., Pütter, C., Kolberg, H.-C., Pott, D., Kurbacher, C., Rezai, M., & Kimmig, R. (2016). Neoadjuvant, anthracycline-free chemotherapy with carboplatin and docetaxel in triple-negative, early-stage breast cancer: a multicentric analysis of rates of pathologic complete response and survival. Journal of Chemotherapy, 28(3), 210–217. https://doi.org/10.1179/1973947815Y.0000000061

94. Roy, V., Pockaj, B. A., Allred, J. B., Apsey, H., Northfelt, D. W., Nikcevich, D., Mattar, B., & Perez, E. A. (2013). A Phase II Trial of Docetaxel and Carboplatin Administered Every 2 Weeks as Preoperative Therapy for Stage II or III Breast Cancer. American Journal of Clinical Oncology, 36(6), 540–544. https://doi.org/10.1097/COC.0b013e318256f619

95. Ando, M., Yamauchi, H., Aogi, K., Shimizu, S., Iwata, H., Masuda, N., Yamamoto, N., Inoue, K., Ohono, S., Kuroi, K., Hamano, T., Sukigara, T., & Fujiwara, Y. (2014). randomised phase II study of weekly paclitaxel with and without carboplatin followed by cyclophosphamide/epirubicin/5-fluorouracil as neoadjuvant chemotherapy for stage II/IIIA breast cancer without HER2 overexpression. Breast Cancer Research and Treatment, 145(2), 401–409. https://doi.org/10.1007/S10549-014-2947-1

96. Sikov, W. M., Berry, D. A., Perou, C. M., Singh, B., Cirrincione, C. T., Tolaney, S. M., Kuzma, C. S., Pluard, T. J., Somlo, G., Port, E. R., Golshan, M., Bellon, J. R., Collyar, D., Hahn, O. M., Carey, L. A., Hudis, C. A., & Winer, E. P. (2015). 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 (Alliance). Journal of Clinical Oncology, 33(1). https://doi.org/10.1200/JCO.2014.57.0572

97. Loibl, S., O'Shaughnessy, J., Untch, M., Sikov, W. M., Rugo, H. S., McKee, M. D., Huober, J., Golshan, M., von Minckwitz, G., Maag, D., Sullivan, D., Wolmark, N., McIntyre, K., Ponce Lorenzo, J. J., Metzger Filho, O., Rastogi, P., Symmans, W. F., Liu, X., & Geyer, C. E. (2018). Addition of the PARP inhibitor veliparib plus carboplatin or carboplatin alone to standard neoadjuvant chemotherapy in triple-negative breast cancer (BrighTNess): a randomised, phase 3 trial. The Lancet Oncology, 19(4), 497–509. https://doi.org/10.1016/S1470-2045(18)30111-6

98. von Minckwitz, G., Schneeweiss, A., Loibl, S., Salat, C., Denkert, C., Rezai, M., Blohmer, J. U., Jackisch, C., Paepke, S., Gerber, B., Zahm, D. M., Kümmel, S., Eidtmann, H., Klare, P., Huober, J., Costa, S., Tesch, H., Hanusch, C., Hilfrich, J., … Untch, M. (2014). Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. The Lancet Oncology, 15(7). https://doi.org/10.1016/S1470-2045(14)70160-3

99. Byrski, T., Huzarski, A. T., Dent, A. R., Gronwald, A. J., Zuziak, A. D., Cybulski, A. C., Kladny, A. J., Gorski, A. B., Lubinski, A. J., & Narod, A. S. A. (n.d.). Response to neoadjuvant therapy with cisplatin in *BRCA*1-positive breast cancer patients. https://doi.org/10.1007/s10549-008-0128-9

100. Tung, N., Arun, B., Hacker, M. R., Hofstatter, E., Toppmeyer, D. L., Isakoff, S. J., Borges, V., Legare, R. D., Isaacs, C., Wolff, A. C., Marcom, P. K., Mayer, E. L., Lange, P. B., Goss, A. J., Jenkins, C., Krop, I. E., Winer, E. P., Schnitt, S. J., & Garber, J. E. (2020). TBCRC 031: randomised Phase II Study of Neoadjuvant Cisplatin Versus Doxorubicin-Cyclophosphamide in Germline *BRCA* Carriers With HER2-Negative Breast Cancer (the INFORM trial). Journal of Clinical Oncology, 38(14), 1539–1548. https://doi.org/10.1200/JCO.19.03292

101. Abraham, J., Vallier, A.-L., Qian, W., Machin, A., Grybowicz, L., Thomas, S., Harvey, C., McAdam, K., Hughes-Davies, L., Roylance, R., Copson, E., Provenzano, E., Pinilla, K., McMurtry, E., Tischkowitz, M., & Earl, H. M. (2018). PARTNER: Randomised, phase II/III trial to evaluate the safety and efficacy of the addition of olaparib to platinum-based neoadjuvant chemotherapy in triple negative and/or germline *BRCA* mutated breast cancer patients. Journal of Clinical Oncology, 36(15_suppl), TPS605–TPS605. https://doi.org/10.1200/JCO.2018.36.15_suppl.TPS605

102. Mayer, I. A., Zhao, F., Arteaga, C. L., Symmans, W. F., Park, B. H., Burnette, B. L., Tevaarwerk, A. J., Garcia, S. F., Smith, K. L., Makower, D. F., Block, M., Morley, K. A., Jani, C. R., Mescher, C., Dewani, S. J., Tawfik, B., Flaum, L. E., Mayer, E. L., Sikov, W. M., … Miller, K. D. (2021). randomised Phase III Postoperative Trial of Platinum-Based Chemotherapy Versus Capecitabine in Patients With Residual Triple-Negative Breast

Cancer Following Neoadjuvant Chemotherapy: ECOG-ACRIN EA1131. Journal of Clinical Oncology, 39(23), 2539–2551. https://doi.org/10.1200/JCO.21.00976758590123456667686901 23

103. Yu, K.-D., Ye, F.-G., He, M., Fan, L., Ma, D., Mo, M., Wu, J., Liu, G.-Y., Di, G.-H., Zeng, X.-H., He, P.-Q., Wu, K.-J., Hou, Y.-F., Wang, J., Wang, C., Zhuang, Z.-G., Song, C.-G., Lin, X.-Y., Toss, A., … Shao, Z.-M. (2020). Effect of Adjuvant Paclitaxel and Carboplatin on Survival in Women With Triple-Negative Breast Cancer. JAMA Oncology, 6(9), 1390. https://doi.org/10.1001/jamaoncol.2020.2965

104. Yee Chung Cheng, Ruth O'Regan, Lubna N Chaudhary, Sailaja Kamaraju, Harvey Einhorn, Emmanuel Sampene, Marialane Pigsley, Mark E Burkard, Christopher R Chitambar, Kari B Wisinski; Abstract PS13-51: A phase II study of neoadjuvant weekly carboplatin/paclitaxel followed by dose-dense doxorubicin/cyclophosphamide (DD AC)in patients with triple negative breast cancer (TNBC): Wisconsin oncology network (WON) study. Cancer Res 15 February 2021; 81 (4_Supplement): PS13–51. https://doi.org/10.1158/1538-7445.SABCS20-PS13-5

105. Caron, M.-C., Sharma, A. K., O'Sullivan, J., Myler, L. R., Ferreira, M. T., Rodrigue, A., Coulombe, Y., Ethier, C., Gagné, J.-P., Langelier, M.-F., Pascal, J. M., Finkelstein, I. J., Hendzel, M. J., Poirier, G. G., & Masson, J.-Y. (2019). Poly(ADP-ribose) polymerase-1 antagonizes DNA resection at double-strand breaks. Nature Communications, 10(1), 2954. https://doi.org/10.1038/s41467-019-10741-9

106. Maya-Mendoza, A., Moudry, P., Merchut-Maya, J. M., Lee, M., Strauss, R., & Bartek, J. (2018). High speed of fork progression induces DNA replication stress and genomic instability. Nature, 559(7713), 279–284. https://doi.org/10.1038/s41586-018-0261-5

107. Farmer, H., McCabe, N., Lord, C. J., Tutt, A. N. J., Johnson, D. A., Richardson, T. B., Santarosa, M., Dillon, K. J., Hickson, I., Knights, C., Martin, N. M. B., Jackson, S. P., Smith, G. C. M., & Ashworth, A. (2005). Targeting the DNA repair defect in *BRCA* mutant cells as a therapeutic strategy. Nature, 434(7035). https://doi.org/10.1038/nature03445

108. Litton, J. K., Rugo, H. S., Ettl, J., Hurvitz, S. A., Gonçalves, A., Lee, K.-H., Fehrenbacher, L., Yerushalmi, R., Mina, L. A., Martin, M., Roché, H., Im, Y.-H., Quek, R. G. W., Markova, D., Tudor, I. C., Hannah, A. L., Eiermann, W., & Blum, J. L. (2018). Talazoparib in Patients with Advanced Breast Cancer and a Germline *BRCA* Mutation. New England Journal of Medicine, 379(8), 753–763. https://doi.org/10.1056/NEJMoa1802905

109. Robson, M., Im, S.-A., Senkus, E., Xu, B., Domchek, S. M., Masuda, N., Delaloge, S., Li, W., Tung, N., Armstrong, A., Wu, W., Goessl, C., Runswick, S., & Conte, P. (2017). Olaparib for Metastatic Breast Cancer in Patients with a Germline *BRCA* Mutation. New England Journal of Medicine, 377(6), 523–533. https://doi.org/10.1056/NEJMoa1706450

110. Eikesdal, H. P., Yndestad, S., Elzawahry, A., Llop-Guevara, A., Gilje, B., Blix, E. S., Espelid, H., Lundgren, S., Geisler, J., Vagstad, G., Venizelos, A., Minsaas, L., Leirvaag, B., Gudlaugsson, E. G., Vintermyr, O. K., Aase, H. S., Aas, T., Balmaña, J., Serra, V., … Lønning, P. E. (2021). Olaparib monotherapy as primary treatment in unselected triple negative breast cancer. Annals of Oncology, 32(2), 240–249. https://doi.org/10.1016/j.annonc.2020.11.009

111. Fasching, P. A., Link, T., Hauke, J., Seither, F., Jackisch, C., Klare, P., Schmatloch, S., Hanusch, C., Huober, J., Stefek, A., Seiler, S., Schmitt, W. D., Uleer, C., Doering, G., Rhiem, K., Schneeweiss, A., Engels, K., Denkert, C., Schmutzler, R. K., … Loibl, & S. (2020). Neoadjuvant paclitaxel/olaparib in comparison to paclitaxel/carboplatinum in patients with HER2-negative breast cancer and homologous recombination deficiency (GeparOLA study) 5. https://doi.org/10.1016/j.annonc.2020.10.471

112. Alba, K. P., McMurtry, E., Vallier, A.-L., Grybowicz, L., Copson, E., Armstrong, A., Roylance, R., Qian, W., Demiris, N., Thomas, S., Harvey, C., Hughes-Davies, L., McAdam, K., del Rosario, P., Harrop, B., Provenzano, E., Tischkowitz, M., Earl, H. M., & Abraham, J. E. (2020). Abstract P3-10-05: Preliminary safety data from stage 1 and 2 of the phase II/III PARTNER trial: Addition of olaparib to platinum-based neoadjuvant chemotherapy in triple negative and/or germline *BRCA* mutated breast cancer patients. Poster Session Abstracts, P3-10-05-P3-10–05. https://doi.org/10.1158/1538-7445.SABCS19-P3-10-05

113. Pusztai, L., Yau, C., Wolf, D. M., Han, H. S., Du, L., Wallace, A. M., String-Reasor, E., Boughey, J. C., Chien, A. J., Elias, A. D., Beckwith, H., Nanda, R., Albain, K. S., Clark, A. S., Kemmer, K., Kalinsky, K., Isaacs, C., Thomas, A., Shatsky, R., … Esserman, L. J. (2021). Durvalumab with olaparib and paclitaxel for high-risk HER2-negative stage II/III breast cancer: Results from the adaptively randomised I-SPY2 trial. Cancer Cell, 39(7), 989-998.e5. https://doi.org/10.1016/j.ccell.2021.05.009

114. Loap, P., Loirat, D., Berger, F., Ricci, F., Vincent-Salomon, A., Ezzili, C., Mosseri, V., Fourquet, A., Ezzalfani, M., & Kirova, Y. (2021). Combination of Olaparib and Radiation Therapy for Triple Negative Breast Cancer: Preliminary Results of the RADIOPARP Phase 1 Trial. International Journal of Radiation Oncology Biology Physics, 109(2), 436–440. https://doi.org/10.1016/j.ijrobp.2020.09.032

115. Litton, J. K., Scoggins, M. E., Hess, K. R., Adrada, B. E., Murthy, R. K., Damodaran, S., DeSnyder, S. M., Brewster, A. M., Barcenas, C. H., Valero, V., Whitman, G. J., Schwartz-Gomez, J., Mittendorf, E. A., Thompson, A. M., Helgason, T., Ibrahim, N., Piwnica-Worms, H., Moulder, S. L., & Arun, B. K. (2020). Neoadjuvant talazoparib for patients with operable breast cancer with a germline *BRCA* pathogenic variant. Journal of Clinical Oncology, 38(5), 388–394. https://doi.org/10.1200/JCO.19.01304

116. Schwab, R., Clark, A. S., Yau, C., Hylton, N., Li, W., Wolfe, D., Chien, A. J., Wallace, A. M., Forero-Torres, A., Stringer-Reasor, E., Nanda, R., Jaskowiak, N., Boughey, J., Haddad, T., Han, H. S., Lee, C., Albain, K., Isaacs, C., Elias, A. D., … Esserman, L. J. (2019). Abstract CT136: Evaluation of talazoparib in combination with irinotecan in early stage, high-risk HER2 negative breast cancer: Results from the I-SPY 2 TRIAL. Clinical Trials, CT136–CT136. https://doi.org/10.1158/1538-7445.AM2019-CT136

117. Severson, T. M., Wolf, D. M., Yau, C., Peeters, J., Wehkam, D., Schouten, P. C., Chin, S. F., Majewski, I. J., Michaut, M., Bosma, A., Pereira, B., Bismeijer, T., Wessels, L., Caldas, C., Bernards, R., Simon, I. M., Glas, A. M., Linn, S., & van't Veer, L. (2017). The *BRCA*1ness signature is associated significantly with response to PARP inhibitor treatment versus control in the I-SPY 2 randomised neoadjuvant setting. Breast Cancer Research, 19(1), 1–9. https://doi.org/10.1186/s13058-017-0861-2

118. Diéras V, Han HS, Kaufman B, et al. Veliparib with carboplatin and paclitaxel in *BRCA*-mutated advanced breast cancer (BROCADE3): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2020;21(10):1269-1282. doi:10.1016/S1470-2045(20)30447-2

119. Jagsi, R., Griffith, K. A., Bellon, J. R., Woodward, W. A., Horton, J. K., Ho, A., Feng, F. Y., Speers, C., Overmoyer, B., Sabel, M., Schott, A. F., & Pierce, L. (2018). Concurrent veliparib with chest wall and nodal radiotherapy in patients with inflammatory or locoregionally recurrent breast cancer: The TBCRC 024 phase I multicenter study. Journal of Clinical Oncology, 36(13), 1317–1322. https://doi.org/10.1200/JCO.2017.77.2665

120. Hahnen, E., Lederer, B., Hauke, J., Loibl, S., Kröber, S., Schneeweiss, A., Denkert, C., Fasching, P. A., Blohmer, J. U., Jackisch, C., Paepke, S., Gerber, B., Kümmel, S., Schem, C., Neidhardt, G., Huober, J., Rhiem, K., Costa, S., Altmüller, J., … Schmutzler, R. K. (2017). Germline Mutation Status, Pathological Complete Response, and Disease-Free Survival in Triple-Negative Breast Cancer. JAMA Oncology, 3(10), 1378. https://doi.org/10.1001/jamaoncol.2017.1007

121. Hoadley, K. A., Powell, B. C., Kanavy, D., Marron, D., Mose, L. E., Hyslop, T., Berry, D. A., Hahn, O., Tolaney, S. M., Sikov, W. M., Perou, C. M., & Carey, L. A. (2020). Abstract P4-05-03: Mutational analysis of triple-negative breast cancer (TNBC): CALGB 40603 (Alliance). Poster Session Abstracts, P4-05-03-P4-05–03. https://doi.org/10.1158/1538-7445.SABCS19-P4-05-03

122. Wolf, D. M., Yau, C., Sanil, A., Glas, A., Petricoin, E., Wulfkuhle, J., Severson, T. M., Linn, S., Brown-Swigart, L., Hirst, G., Buxton, M., DeMichele, A., Hylton, N., Symmans, F., Yee, D., Paoloni, M., Esserman, L., Berry, D., Rugo, H., … van 't Veer, L. (2017). DNA repair deficiency biomarkers and the 70-gene ultra-high risk signature as predictors of veliparib/carboplatin response in the I-SPY 2 breast cancer trial. Npj Breast Cancer, 3(1), 1–8. https://doi.org/10.1038/s41523-017-0025-7

123. Schneeweiss, A., Möbus, V., Tesch, H., Hanusch, C., Denkert, C., Lübbe, K., Huober, J., Klare, P., Kümmel, S., Untch, M., Kast, K., Jackisch, C., Thomalla, J., Ingold-Heppner, B., Blohmer, J.-U., Rezai, M., Frank, M., Engels, K., Rhiem, K., … Loibl, S. (2019). Intense dose-dense epirubicin, paclitaxel, cyclophosphamide versus weekly paclitaxel, liposomal doxorubicin (plus carboplatin in triple-negative breast cancer) for neoadjuvant treatment of high-risk early breast cancer (GeparOcto—GBG 84): A randomised phase III trial. European Journal of Cancer, 106, 181–192. https://doi.org/10.1016/j.ejca.2018.10.015

124. Graeser, M., McCarthy, A., Lord, C. J., Savage, K., Hills, M., Salter, J., Orr, N., Parton, M., Smith, I. E., Reis-Filho, J. S., Dowsett, M., Ashworth, A., & Turner, N. C. (2010). A Marker of Homologous Recombination Predicts Pathologic Complete Response to Neoadjuvant Chemotherapy in Primary Breast Cancer. Clinical Cancer Research, 16(24), 6159–6168. https://doi.org/10.1158/1078-0432.CCR-10-1027

125. Peng, G., Chun-Jen Lin, C., Mo, W., Dai, H., Park, Y.-Y., Kim, S. M., Peng, Y., Mo, Q., Siwko, S., Hu, R., Lee, J.-S., Hennessy, B., Hanash, S., Mills, G. B., & Lin, S.-Y. (2014). Genome-wide transcriptome profiling of homologous recombination DNA repair. Nature Communications, 5(1), 3361. https://doi.org/10.1038/ncomms4361

126. Vollebergh, M. A., Lips, E. H., Nederlof, P. M., Wessels, L. F., Wesseling, J., vd Vijver, M. J., de Vries, E. G., van Tinteren, H., Jonkers, J., Hauptmann, M., Rodenhuis, S., & Linn, S. C. (2014). Genomic patterns resembling *BRCA*1- and *BRCA*2-mutated breast cancers predict benefit of intensified carboplatin-based chemotherapy. Breast Cancer Research, 16(3), R47. https://doi.org/10.1186/bcr3655

127. Telli, M. L., Jensen, K. C., Vinayak, S., Kurian, A. W., Lipson, J. A., Flaherty, P. J., Timms, K., Abkevich, V., Schackmann, E. A., Wapnir, I. L., Carlson, R. W., Chang, P.-J., Sparano, J. A., Head, B., Goldstein, L. J., Haley, B., Dakhil, S. R., Reid, J. E., Hartman, A.-R., … Ford, J. M. (2015). Phase II Study of Gemcitabine, Carboplatin, and Iniparib As Neoadjuvant Therapy for Triple-Negative and *BRCA*1 / 2 Mutation–Associated Breast Cancer With Assessment of a tumour-Based Measure of Genomic Instability: PrECOG 0105. Journal of Clinical Oncology, 33(17), 1895–1901. https://doi.org/10.1200/JCO.2014.57.0085

128. Loibl, S., Weber, K. E., Timms, K. M., Elkin, E. P., Hahnen, E., Fasching, P. A., Lederer, B., Denkert, C., Schneeweiss, A., Braun, S., Salat, C. T., Rezai, M., Blohmer, J. U., Zahm, D. M., Jackisch, C., Gerber, B., Klare, P., Kümmel, S., Schem, C., … Untch, M. (2018). Survival analysis of carboplatin added to an anthracycline/taxane-based neoadjuvant chemotherapy and HRD score as predictor of response—final results from GeparSixto. Annals of Oncology, 29(12), 2341–2347. https://doi.org/10.1093/annonc/mdy460

129. von Minckwitz, G., Timms, K., Untch, M., Elkin, E. P., Fasching, P. A., Schneeweiss, A., Salat, C., Rezai, M., Blohmer, J. U., Zahm, D. M., Jackisch, C., Gerber, B., Klare, P., Kümmel, S., Eidtmann, H., Paepke, S., Reid, J. E., Nekljudova, V., Hartman, A.-R., & Loibl, S. (2015). Prediction of pathological complete response (pCR) by Homologous Recombination Deficiency (HRD) after carboplatin-containing neoadjuvant chemotherapy in patients with TNBC: Results from GeparSixto. Journal of Clinical Oncology, 33(15_suppl), 1004–1004. https://doi.org/10.1200/jco.2015.33.15_suppl.1004

130. Chopra, N., Tovey, H., Pearson, A., Cutts, R., Toms, C., Proszek, P., Hubank, M., Dowsett, M., Dodson, A., Daley, F., Kriplani, D., Gevensleben, H., Davies, H. R., Degasperi, A., Roylance, R., Chan, S., Tutt, A., Skene, A., Evans, A., … Turner, N. C. (2020). Homologous recombination DNA repair deficiency and PARP inhibition activity in primary triple negative breast cancer. Nature Communications, 11(1), 2662. https://doi.org/10.1038/s41467-020-16142-7

131. Prat, A., Lluch, A., Albanell, J., Barry, W. T., Fan, C., Chacón, J. I., et al. (2014). Predicting response and survival in chemotherapy-treated triple-negative breast cancer. British Journal of Cancer 111, 1532–1541. doi:10.1038/bjc.2014.444.

132. Sohn, I., Jung, W. Y., & Sung, C. O. (2012). Somatic hypermutation and outcomes of platinum based chemotherapy in patients with high grade serous ovarian cancer. Gynecologic Oncology, 126(1), 103–108. https://doi.org/10.1016/j.ygyno.2012.03.050

133. Mei, P., Freitag, C. E., Wei, L., Zhang, Y., Parwani, A. v., & Li, Z. (2020). High tumour mutation burden is associated with DNA damage repair gene mutation in breast carcinomas. Diagnostic Pathology, 15(1), 50. https://doi.org/10.1186/s13000-020-00971-7

134. Gatti-Mays, M. E., Balko, J. M., Gameiro, S. R., Bear, H. D., Prabhakaran, S., Fukui, J., Disis, M. L., Nanda, R., Gulley, J. L., Kalinsky, K., Abdul Sater, H., Sparano, J. A., Cescon, D., Page, D. B., McArthur, H., Adams, S., & Mittendorf, E. A. (2019). If we build it they will come: targeting the immune response to breast cancer. Npj Breast Cancer, 5(1), 37. https://doi.org/10.1038/s41523-019-0133-7

135. Mittendorf, E. A., Philips, A. v., Meric-Bernstam, F., Qiao, N., Wu, Y., Harrington, S., Su, X., Wang, Y., Gonzalez-Angulo, A. M., Akcakanat, A., Chawla, A., Curran, M., Hwu, P., Sharma, P., Litton, J. K., Molldrem, J. J., & Alatrash, G. (2014). PD-L1 Expression in Triple-Negative Breast Cancer. Cancer Immunology Research, 2(4), 361–370. https://doi.org/10.1158/2326-6066.CIR-13-0127

136. Nanda, R., Liu, M. C., Yau, C., Shatsky, R., Pusztai, L., Wallace, A., Chien, A. J., Forero-Torres, A., Ellis, E., Han, H., Clark, A., Albain, K., Boughey, J. C., Jaskowiak, N. T., Elias, A., Isaacs, C., Kemmer, K., Helsten, T., Majure, M., … Esserman, L. J. (2020). Effect of Pembrolizumab Plus Neoadjuvant Chemotherapy on Pathologic Complete Response in Women With Early-Stage Breast Cancer. JAMA Oncology, 6(5), 676. https://doi.org/10.1001/jamaoncol.2019.6650

137. Schmid, P., Cortes, J., Pusztai, L., McArthur, H., Kümmel, S., Bergh, J., Denkert, C., Park, Y. H., Hui, R., Harbeck, N., Takahashi, M., Foukakis, T., Fasching, P. A., Cardoso, F., Untch, M., Jia, L., Karantza, V., Zhao, J., Aktan, G., … O'Shaughnessy, J. (2020). Pembrolizumab for Early Triple-Negative Breast Cancer. New England Journal of Medicine, 382(9), 810–821. https://doi.org/10.1056/NEJMoa1910549

138. Tarantino, P., Corti, C., Schmid, P. et al. Immunotherapy for early triple negative breast cancer: research agenda for the next decade. npj Breast Cancer 8, 23 (2022). https://doi.org/10.1038/s41523-022-00386-1

139. Voorwerk, L., Slagter, M., Horlings, H.M. et al. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. Nat Med 25, 920–928 (2019). https://doi.org/10.1038/s41591-019-0432-4

140. Gianni L, Huang CS, Egle D, et al. Pathologic complete response (pCR) to neoadjuvant treatment with or without atezolizumab in triple-negative, early high-risk and locally advanced breast cancer: NeoTRIP Michelangelo randomized study [published online ahead of print, 2022 Feb 17]. Ann Oncol. 2022;S0923-7534(22)00113-2. doi:10.1016/j.annonc.2022.02.004

141. Loibl, S., Untch, M., Burchardi, N., Huober, J., Sinn, B. V., Blohmer, J.-U., Grischke, E.-M., Furlanetto, J., Tesch, H., Hanusch, C., Engels, K., Rezai, M., Jackisch, C., Schmitt, W. D., von Minckwitz, G., Thomalla, J., Kümmel, S., Rautenberg, B., Fasching, P. A., … Schneeweiss, A. (2019). A randomised phase II study investigating durvalumab in addition to an anthracycline taxane-based neoadjuvant therapy in early triple-negative breast cancer: clinical results and biomarker analysis of GeparNuevo study. Annals of Oncology, 30(8), 1279–1288. https://doi.org/10.1093/annonc/mdz158

142. Loibl, S., Schneeweiss, A., Huober, J. B., Braun, M., Rey, J., Blohmer, J. U., Furlanetto, J., Zahm, D. M., Hanusch, C., Thomalla, J., Jackisch, C., Staib, P., Link, T., Rhiem, K., Solbach, C., Fasching, P. A., Burchardi, N., Denkert, C., & Untch, M. (2021). Durvalumab improves long-term outcome in TNBC: results from the phase II randomised GeparNUEVO study investigating neodjuvant durvalumab in addition to an anthracycline/taxane based neoadjuvant chemotherapy in early triple-negative breast cancer (TNBC). Journal of Clinical Oncology, 39(15_suppl), 506–506. https://doi.org/10.1200/JCO.2021.39.15_suppl.506

143. Brown, L. C., and Loi, S. (2022). Immune checkpoint inhibition in the treatment of early stage triple negative breast cancer: 2021 update. The Breast 0. doi:10.1016/J.BREAST.2021.12.018.

144. Yee, D., DeMichele, A. M., Yau, C., Isaacs, C., Symmans, W. F., Albain, K. S., Chen, Y.-Y., Krings, G., Wei, S., Harada, S., Datnow, B., Fadare, O., Klein, M., Pambuccian, S., Chen, B., Adamson, K., Sams, S., Mhawech-Fauceglia, P., Magliocco, A., … Berry, D. A. (2020). Association of Event-Free and Distant Recurrence–Free Survival With Individual-Level Pathologic Complete Response in Neoadjuvant Treatment of Stages 2 and 3 Breast Cancer. JAMA Oncology, 6(9). https://doi.org/10.1001/jamaoncol.2020.2535

145. Ademuyiwa, F. O., Gao, F., Chen, I., Northfelt, D. W., Wesolowski, R., Arora, M., Brufsky, A., Dees, C., Santa-Maria, C. A., Connolly, R. M., Force, J., Moreno-Aspitia, A., Larson, S., Sharon, E., & Gillanders, W. (2021). Abstract PD14-09: Nci 10013 - A randomised phase 2 study of neoadjuvant carboplatin and paclitaxel, with or without atezolizumab in triple negative breast cancer (TNBC). Poster Spotlight Session Abstracts, PD14-09-PD14-09. https://doi.org/10.1158/1538-7445.SABCS20-PD14-09

146. Yam, C., Mittendorf, E. A., Sun, R., Huo, L., Damodaran, S., Rauch, G. M., Candelaria, R. P., Adrada, B. E., Seth, S., Symmans, W. F., Murthy, R. K., White, J. B., Ravenberg, E., Clayborn, A., Prabhakaran, S., Valero, V., Thompson, A. M., Tripathy, D., Moulder, S. L., & Litton, J. K. (2021). Neoadjuvant atezolizumab (atezo) and nab-paclitaxel (nab-p) in patients (pts) with triple-negative breast cancer (TNBC) with suboptimal clinical response to doxorubicin and cyclophosphamide (AC). Journal of Clinical Oncology, 39(15_suppl), 592–592. https://doi.org/10.1200/JCO.2021.39.15_suppl.592

147. Foldi, J., Silber, A., Reisenbichler, E. et al. Neoadjuvant durvalumab plus weekly nab-paclitaxel and dose-dense doxorubicin/cyclophosphamide in triple-negative breast cancer. npj Breast Cancer 7, 9 (2021). https://doi.org/10.1038/s41523-021-00219-7

148. Rugo, H. S., Loi, S., Adams, S., Schmid, P., Schneeweiss, A., Barrios, C. H., Iwata, H., Diéras, V., Winer, E. P., Kockx, M. M., Peeters, D., Chui, S. Y., Lin, J. C., Nguyen-Duc, A., Viale, G., Molinero, L., & Emens, L. A. (2021). PD-L1 Immunohistochemistry Assay Comparison in Atezolizumab Plus nab -Paclitaxel–Treated Advanced Triple-Negative Breast Cancer. JNCI: Journal of the National Cancer Institute, 113(12), 1733–1743. https://doi.org/10.1093/jnci/djab108

149. Wei, S. C., Duffy, C. R., & Allison, J. P. (2018). Fundamental Mechanisms of Immune Checkpoint Blockade Therapy. Cancer Discovery, 8(9), 1069–1086. https://doi.org/10.1158/2159-8290.CD-18-0367

150. Sha, D., Jin, Z., Budczies, J., Kluck, K., Stenzinger, A., & Sinicrope, F. A. (2020). tumour mutational burden as a predictive biomarker in solid tumours. Cancer Discovery, 10(12), 1808–1825. https://doi.org/10.1158/2159-8290.CD-20-0522

151. Cristescu, R., Mogg, R., Ayers, M., Albright, A., Murphy, E., Yearley, J., Sher, X., Liu, X. Q., Lu, H., Nebozhyn, M., Zhang, C., Lunceford, J. K., Joe, A., Cheng, J., Webber, A. L., Ibrahim, N., Plimack, E. R., Ott, P. A., Seiwert, T. Y., … Kaufman, D. (2018). Pan-tumour genomic biomarkers for PD-1 checkpoint blockade-based immunotherapy. Science, 362(6411). https://doi.org/10.1126/science.aar3593

152. Yarchoan, M., Albacker, L. A., Hopkins, A. C., Montesion, M., Murugesan, K., Vithayathil, T. T., Zaidi, N., Azad, N. S., Laheru, D. A., Frampton, G. M., & Jaffee, E. M. (2019). PD-L1 expression and tumour mutational burden are independent biomarkers in most cancers. JCI Insight, 4(6). https://doi.org/10.1172/jci.insight.126908

153. Marabelle, A., Fakih, M., Lopez, J., Shah, M., Shapira-Frommer, R., Nakagawa, K., Chung, H. C., Kindler, H. L., Lopez-Martin, J. A., Miller, W. H., Italiano, A., Kao, S., Piha-Paul, S. A., Delord, J.-P., McWilliams, R. R., Fabrizio, D. A., Aurora-Garg, D., Xu, L., Jin, F., … Bang, Y.-J. (2020). Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. The Lancet Oncology, 21(10), 1353–1365. https://doi.org/10.1016/S1470-2045(20)30445-9

154. Criscitiello, C., Bayar, M. A., Curigliano, G., Symmans, F. W., Desmedt, C., Bonnefoi, H., Sinn, B., Pruneri, G., Vicier, C., Pierga, J. Y., Denkert, C., Loibl, S., Sotiriou, C., Michiels, S., & André, F. (2018). A gene signature to predict high tumour-infiltrating lymphocytes after neoadjuvant chemotherapy and outcome in patients with triple-negative breast cancer. Annals of Oncology : Official Journal of the European Society for Medical Oncology, 29(1), 162–169. https://doi.org/10.1093/ANNONC/MDX691

155. McGrail, D. J., Pilié, P. G., Rashid, N. U., Voorwerk, L., Slagter, M., Kok, M., Jonasch, E., Khasraw, M., Heimberger, A. B., Lim, B., Ueno, N. T., Litton, J. K., Ferrarotto, R., Chang, J. T., Moulder, S. L., & Lin, S. Y. (2021). High tumour mutation burden fails to predict immune checkpoint blockade response across all cancer types. Annals of Oncology, 32(5), 661–672. https://doi.org/10.1016/j.annonc.2021.02.006

156. Gao, G., Wang, Z., Qu, X., & Zhang, Z. (2020). Prognostic value of tumour-infiltrating lymphocytes in patients with triple-negative breast cancer: A systematic review and meta-analysis. BMC Cancer, 20(1), 1–15. https://doi.org/10.1186/S12885-020-6668-Z/FIGURES/6

157. Loi, S., Drubay, D., Adams, S., Pruneri, G., Francis, P. A., Lacroix-Triki, M., Joensuu, H., Dieci, M. V., Badve, S., Demaria, S., Gray, R., Munzone, E., Lemonnier, J., Sotiriou, C., Piccart, M. J., Kellokumpu-Lehtinen, P. L., Vingiani, A., Gray, K., Andre, F., … Michiels, S. (2019). tumour-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Early-Stage Triple-Negative Breast Cancers. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, 37(7), 559–569. https://doi.org/10.1200/JCO.18.01010

158. Gruosso, T., Gigoux, M., Manem, V. S. K., Bertos, N., Zuo, D., Perlitch, I., Saleh, S. M. I., Zhao, H., Souleimanova, M., Johnson, R. M., Monette, A., Ramos, V. M., Hallett, M. T., Stagg, J., Lapointe, R., Omeroglu, A., Meterissian, S., Buisseret, L., van den Eynden, G., … Park, M. (2019). Spatially distinct tumour immune microenvironments stratify triple-negative breast cancers. The Journal of Clinical Investigation, 129(4), 1785–1800. https://doi.org/10.1172/JCI96313

159. Pérez-Pena, J., Tibor Fekete, J., Páez, R., Baliu-Piqué, M., García-Saenz, J. Á., García-Barberán, V., Manzano, A., Pérez-Segura, P., Esparis-Ogando, A., Pandiella, A., Gyorffy, B., & Ocana, A. (2019). A Transcriptomic Immunologic Signature Predicts Favorable Outcome in Neoadjuvant Chemotherapy Treated Triple Negative Breast tumours. Frontiers in Immunology, 10, 2802. https://doi.org/10.3389/FIMMU.2019.02802/BIBTEX

160. Craven, K. E., Gökmen-Polar, Y., & Badve, S. S. (2021). CIBERSORT analysis of TCGA and METABRIC identifies subgroups with better outcomes in triple negative breast cancer. Scientific Reports 2021 11:1, 11(1), 1–19. https://doi.org/10.1038/s41598-021-83913-7

161. Callari, M., Cappelletti, V., D'Aiuto, F., Musella, V., Lembo, A., Petel, F., Karn, T., Iwamoto, T., Provero, P., Daidone, M. G., Gianni, L., & Bianchini, G. (2016). Subtype-Specific Metagene-Based Prediction of Outcome after Neoadjuvant and Adjuvant Treatment in Breast Cancer. Clinical Cancer Research, 22(2), 337–345. https://doi.org/10.1158/1078-0432.CCR-15-0757

162. Sharma, P., Barlow, W. E., Godwin, A. K., Parkes, E. E., Knight, L. A., Walker, S. M., Kennedy, R. D., Harkin, D. P., Logan, G. E., Steele, C. J., Lambe, S. M., Badve, S., Gökmen-Polar, Y., Pathak, H. B., Isakova, K., Linden, H. M., Porter, P., Pusztai, L., Thompson, A. M., … Hayes, D. F. (2019). Validation of the DNA Damage Immune Response Signature in Patients With Triple-Negative Breast Cancer From the SWOG 9313c Trial. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, 37(36), 3484–3492. https://doi.org/10.1200/JCO.19.00693

163. Lv, Y., Lv, D., Lv, X., Xing, P., Zhang, J., & Zhang, Y. (2021). Immune Cell Infiltration-Based Characterization of Triple-Negative Breast Cancer Predicts Prognosis and Chemotherapy Response Markers. Frontiers in Genetics, 12, 354. https://doi.org/10.3389/FGENE.2021.616469/BIBTEX

164. Loibl, S., Sinn, B., Karn, T., Untch, M., Treue, D., Sinn, H.-P., Weber, K., Hanusch, C., Fasching, P., Huober, J., Zahm, D.-M., Jackisch, C., Thomalla, J., Blohmer, J.-U., Marmé, F., Klauschen, F., Rhiem, K., Felder, B., von Minckwitz, G., … Denkert, C. (2019). Abstract PD2-07: mRNA signatures predict response to durvalumab therapy in triple negative breast cancer (TNBC)– Results of the translational biomarker programme of the neoadjuvant double-blind placebo controlled GeparNuevo trial. Poster Discussion Abstracts, PD2-07-PD2-07. https://doi.org/10.1158/1538-7445.SABCS18-PD2-07

165. Marcus, L., Lemery, S. J., Keegan, P., & Pazdur, R. (2019). FDA Approval Summary: Pembrolizumab for the Treatment of Microsatellite Instability-High Solid tumours. Clinical Cancer Research : An Official Journal of the American Association for Cancer Research, 25(13), 3753–3758. https://doi.org/10.1158/1078-0432.CCR-18-4070

166. Zou, X., Koh, G. C. C., Nanda, A. S., Degasperi, A., Urgo, K., Roumeliotis, T. I., Agu, C. A., Badja, C., Momen, S., Young, J., Amarante, T. D., Side, L., Brice, G., Perez-Alonso, V., Rueda, D., Gomez, C., Bushell, W., Harris, R., Choudhary, J. S., … Nik-Zainal, S. (2021). A systematic CRISPR screen defines mutational mechanisms underpinning signatures caused by replication errors and endogenous DNA damage. Nature Cancer, 2(6), 643–657. https://doi.org/10.1038/S43018-021-00200-0

167. Martín, M., Chan, A., Dirix, L., O'Shaughnessy, J., Hegg, R., Manikhas, A., Shtivelband, M., Krivorotko, P., Batista López, N., Campone, M., Ruiz Borrego, M., Khan, Q. J., Beck, J. T., Ramos Vázquez, M., Urban, P., Goteti, S., di Tomaso, E., Massacesi, C., & Delaloge, S. (2017). A randomised adaptive phase II/III study of buparlisib, a pan-class I PI3K inhibitor, combined with paclitaxel for the treatment of HER2- advanced breast cancer (BELLE-4). Annals of Oncology : Official Journal of the European Society for Medical Oncology, 28(2), 313–320. https://doi.org/10.1093/ANNONC/MDW562

168. Oliveira, M., Saura, C., Nuciforo, P., Calvo, I., Andersen, J., Passos-Coelho, J. L., Gil Gil, M., Bermejo, B., Patt, D. A., Ciruelos, E., de La Peña, L., Xu, N., Wongchenko, M., Shi, Z., Singel, S. M., & Isakoff, S. J. (2019). FAIRLANE, a double-blind placebo-controlled randomised phase II trial of neoadjuvant ipatasertib plus paclitaxel for early triple-negative breast cancer. Annals of Oncology : Official Journal of the European Society for Medical Oncology, 30(8), 1289–1297. https://doi.org/10.1093/ANNONC/MDZ177

169. Chien, A. J., Tripathy, D., Albain, K. S., Symmans, W. F., Rugo, H. S., Melisko, M. E., Wallace, A. M., Schwab, R., Helsten, T., Forero-Torres, A., Stringer-Reasor, E., Ellis, E. D., Kaplan, H. G., Nanda, R., Jaskowiak, N., Murthy, R., Godellas, C., Boughey, J. C., Elias, A. D., … Esserman, L. J. (2020). MK-2206 and Standard Neoadjuvant Chemotherapy Improves Response in Patients With Human Epidermal Growth Factor Receptor 2-Positive and/or Hormone Receptor-Negative Breast Cancers in the I-SPY 2 Trial. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, 38(10), 1059–1069. https://doi.org/10.1200/JCO.19.01027

170. Gonzalez-Angulo, A. M., Akcakanat, A., Liu, S., Green, M. C., Murray, J. L., Chen, H., Palla, S. L., Koenig, K. B., Brewster, A. M., Valero, V., Ibrahim, N. K., Moulder-Thompson, S., Litton, J. K., Tarco, E., Moore, J., Flores, P., Crawford, D., Dryden, M. J., Symmans, W. F., … Meric-Bernstam, F. (2014). Open-label randomised clinical trial of standard neoadjuvant chemotherapy with paclitaxel followed by FEC versus the combination of paclitaxel and everolimus followed by FEC in women with triple receptor-negative breast cancer†. Annals of Oncology : Official Journal of the European Society for Medical Oncology, 25(6), 1122–1127. https://doi.org/10.1093/ANNONC/MDU124

171. Park, S., Koo, J., Park, H. S., Kim, J. H., Choi, S. Y., Lee, J. H., Park, B. W., & Lee, K. S. (2010). Expression of androgen receptors in primary breast cancer. Annals of Oncology : Official Journal of the European Society for Medical Oncology, 21(3), 488–492. https://doi.org/10.1093/ANNONC/MDP510

172. Niemeier, L. A., Dabbs, D. J., Beriwal, S., Striebel, J. M., & Bhargava, R. (2010). Androgen receptor in breast cancer: expression in oestrogen receptor-positive tumours and in oestrogen receptor-negative tumours with apocrine differentiation. Modern Pathology : An Official Journal of the United States and Canadian Academy of Pathology, Inc, 23(2), 205–212. https://doi.org/10.1038/MODPATHOL.2009.159

173. Traina, T. A., Miller, K., Yardley, D. A., Eakle, J., Schwartzberg, L. S., O'Shaughnessy, J., Gradishar, W., Schmid, P., Winer, E., Kelly, C., Nanda, R., Gucalp, A., Awada, A., Garcia-Estevez, L., Trudeau, M. E., Steinberg, J., Uppal, H., Tudor, I. C., Peterson, A., & Cortes, J. (2018). Enzalutamide for the Treatment of Androgen Receptor-Expressing Triple-Negative Breast Cancer. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, 36(9), 884–890. https://doi.org/10.1200/JCO.2016.71.3495

174. Lehmann, B. D., Abramson, V. G., Sanders, M. E., Mayer, E. L., Haddad, T. C., Nanda, R., van Poznak, C., Storniolo, A. M., Nangia, J. R., Gonzalez-Ericsson, P. I., Sanchez, V., Johnson, K. N., Abramson, R. G., Chen, S. C., Shyr, Y., Arteaga, C. L., Wolff, A. C., & Pietenpol, J. A. (2020). TBCRC 032 IB/II Multicenter Study: Molecular Insights to AR Antagonist and PI3K Inhibitor Efficacy in Patients with AR + Metastatic Triple-Negative Breast Cancer. Clinical Cancer Research : An Official Journal of the American Association for Cancer Research, 26(9), 2111–2123. https://doi.org/10.1158/1078-0432.CCR-19-2170

175. Bonnefoi, H., Grellety, T., Tredan, O., Saghatchian, M., Dalenc, F., Mailliez, A., L'Haridon, T., Cottu, P., Abadie-Lacourtoisie, S., You, B., Mousseau, M., Dauba, J., del Piano, F., Desmoulins, I., Coussy, F., Madranges, N., Grenier, J., Bidard, F. C., Proudhon, C., … Gonçalves, A. (2016). 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). Annals of Oncology : Official Journal of the European Society for Medical Oncology, 27(5), 812–818. https://doi.org/10.1093/ANNONC/MDW067

176. Gucalp, A., Tolaney, S., Isakoff, S. J., Ingle, J. N., Liu, M. C., Carey, L. A., Blackwell, K., Rugo, H., Nabell, L., Forero, A., Stearns, V., Doane, A. S., Danso, M., Moynahan, M. E., Momen, L. F., Gonzalez, J. M., Akhtar, A., Giri, D. D., Patil, S., … Traina, T. A. (2013). Phase II trial of bicalutamide in patients with androgen receptor-positive, oestrogen receptor-negative metastatic Breast Cancer. Clinical Cancer Research : An Official Journal of the American Association for Cancer Research, 19(19), 5505–5512. https://doi.org/10.1158/1078-0432.CCR-12-3327

177. Wulfkuhle, J. D., Yau, C., Wolf, D. M., Vis, D. J., Gallagher, R. I., Brown-Swigart, L., Hirst, G., Voest, E. E., DeMichele, A., Hylton, N., Symmans, F., Yee, D., Esserman, L., Berry, D., Liu, M., Park, J. W., Wessels, L. F. A., Veer, L. van't, & III, E. F. P. (2018). Evaluation of the HER/PI3K/AKT Family Signaling Network as a Predictive Biomarker of Pathologic Complete Response for Patients With Breast Cancer Treated With Neratinib in the I-SPY 2 TRIAL. Https://Doi.Org/10.1200/PO.18.00024, 2, 1–20. https://doi.org/10.1200/PO.18.00024

178. Schettini, F., Chic, N., Brasó-Maristany, F., Paré, L., Pascual, T., Conte, B., Martínez-Sáez, O., Adamo, B., Vidal, M., Barnadas, E., Fernández-Martinez, A., González-Farre, B., Sanfeliu, E., Cejalvo, J. M., Perrone, G., Sabarese, G., Zalfa, F., Peg, V., Fasani, R., … Prat, A. (2021). Clinical, pathological, and PAM50 gene expression features of HER2-low breast cancer. Npj Breast Cancer 2021 7:1, 7(1), 1–13. https://doi.org/10.1038/s41523-020-00208-2

179. Conlon, N. T., Kooijman, J. J., van Gerwen, S. J. C., Mulder, W. R., Zaman, G. J. R., Diala, I., Eli, L. D., Lalani, A. S., Crown, J., & Collins, D. M. (2021). Comparative analysis of drug response and gene profiling of HER2-targeted tyrosine kinase inhibitors. British Journal of Cancer 2021 124:7, 124(7), 1249–1259. https://doi.org/10.1038/s41416-020-01257-x

180. Wulfkuhle, J. D., Yau, C., Wolf, D. M., Gallagher, R. I., Deng, J., Swigart, L. B., Hirst, G., Liu, M. C., Park, J. W., Esserman, L., Berry, D. A., Veer, L. van't, & Petricoin, E. (2015). Protein activation mapping and exploratory predictive markers for pCR in triple-negative breast cancer patients treated with neratinib in the I-SPY 2 TRIAL. https://doi.org/10.1200/JCO.2015.33.15_SUPPL.1085

181. Modi, S., Park, H., Murthy, R. K., Iwata, H., Tamura, K., Tsurutani, J., Moreno-Aspitia, A., Doi, T., Sagara, Y., Redfern, C., Krop, I. E., Lee, C., Fujisaki, Y., Sugihara, M., Zhang, L., Shahidi, J., & Takahashi, S. (2020). Antitumour Activity and Safety of Trastuzumab Deruxtecan in Patients With HER2-Low-Expressing Advanced Breast Cancer: Results From a Phase Ib Study. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, 38(17), 1887–1896. https://doi.org/10.1200/JCO.19.02318

182. Banerji, U., van Herpen, C. M. L., Saura, C., Thistlethwaite, F., Lord, S., Moreno, V., Macpherson, I. R., Boni, V., Rolfo, C., de Vries, E. G. E., Rottey, S., Geenen, J., Eskens, F., Gil-Martin, M., Mommers, E. C., Koper, N. P., & Aftimos, P. (2019). Trastuzumab duocarmazine in locally advanced and metastatic solid tumours and HER2-expressing breast cancer: a phase 1 dose-escalation and dose-expansion study. The Lancet. Oncology, 20(8), 1124–1135. https://doi.org/10.1016/S1470-2045(19)30328-6

183. Linderholm, B. K., Hellborg, H., Johansson, U., Elmberger, G., Skoog, L., Lehtiö, J., & Lewensohn, R. (2009). Significantly higher levels of vascular endothelial growth factor (VEGF) and shorter survival times for patients with primary operable triple-negative breast cancer. Annals of Oncology : Official Journal of the European Society for Medical Oncology, 20(10), 1639–1646. https://doi.org/10.1093/ANNONC/MDP062

184. Ali, E. M., Sheta, M., & el Mohsen, M. A. (2011). Elevated serum and tissue VEGF associated with poor outcome in breast cancer patients. Alexandria Journal of Medicine, 47(3), 217–224. https://doi.org/10.1016/j.ajme.2011.07.003

185. von Minckwitz, G., Eidtmann, H., Rezai, M., Fasching, P. A., Tesch, H., Eggemann, H., Schrader, I., Kittel, K., Hanusch, C., Kreienberg, R., Solbach, C., Gerber, B., Jackisch, C., Kunz, G., Blohmer, J.-U., Huober, J., Hauschild, M., Fehm, T., Müller, B. M., … Untch, M. (2012). Neoadjuvant Chemotherapy and Bevacizumab for HER2-Negative Breast Cancer. New England Journal of Medicine, 366(4), 299–309. https://doi.org/10.1056/NEJMOA1111065/SUPPL_FILE/NEJMOA1111065_DISCLOSURES.PDF

186. Bear, H. D., Tang, G., Rastogi, P., Geyer, C. E., Liu, Q., Robidoux, A., Baez-Diaz, L., Brufsky, A. M., Mehta, R. S., Fehrenbacher, L., Young, J. A., Senecal, F. M., Gaur, R., Margolese, R. G., Adams, P. T., Gross, H. M., Costantino, J. P., Paik, S., Swain, S. M., … Wolmark, N. (2015). Neoadjuvant plus adjuvant bevacizumab in early breast cancer (NSABP B-40 [NRG Oncology]): secondary outcomes of a phase 3, randomised controlled trial. The Lancet. Oncology, 16(9), 1037–1048. https://doi.org/10.1016/S1470-2045(15)00041-8

187. Cameron, D., Brown, J., Dent, R., Jackisch, C., Mackey, J., Pivot, X., Steger, G. G., Suter, T. M., Toi, M., Parmar, M., Laeufle, R., Im, Y. H., Romieu, G., Harvey, V., Lipatov, O., Pienkowski, T., Cottu, P., Chan, A., Im, S. A., … Bell, R. (2013). Adjuvant bevacizumab-containing therapy in triple-negative breast cancer (BEATRICE): primary results of a randomised, phase 3 trial. The Lancet. Oncology, 14(10), 933–942. https://doi.org/10.1016/S1470-2045(13)70335-8

188. Earl, H. M., Hiller, L., Dunn, J. A., Blenkinsop, C., Grybowicz, L., Vallier, A.-L., Abraham, J., Thomas, J., Provenzano, E., Hughes-Davies, L., Gounaris, I., McAdam, K., Chan, S., Ahmad, R., Hickish, T., Houston, S., Rea, D., Bartlett, J., Caldas, C., … Hayward, L. (2015). Efficacy of neoadjuvant bevacizumab added to docetaxel followed by fluorouracil, epirubicin, and cyclophosphamide, for women with HER2-negative early breast cancer (ARTemis): an open-label, randomised, phase 3 trial. The Lancet Oncology, 16(6), 656–666. https://doi.org/10.1016/S1470-2045(15)70137-3

189. Babina, I. S., & Turner, N. C. (2017). Advances and challenges in targeting FGFR signalling in cancer. Nature Reviews. Cancer, 17(5), 318–332. https://doi.org/10.1038/NRC.2017.8

190. Sung, V. Y. C., Knight, J. F., Johnson, R. M., Stern, Y. E., Saleh, S. M., Savage, P., Monast, A., Zuo, D., Duhamel, S., & Park, M. (2021). Co-dependency for MET and FGFR1 in basal triple-negative breast cancers. Npj Breast Cancer 2021 7:1, 7(1), 1–15. https://doi.org/10.1038/s41523-021-00238-4

191. Williams, C. B., Soloff, A. C., Ethier, S. P., & Yeh, E. S. (2015). Perspectives on Epidermal Growth Factor Receptor Regulation in Triple-Negative Breast Cancer (pp. 253–281). https://doi.org/10.1016/bs.acr.2015.04.008

192. Lehmann, B. D., Bauer, J. A., Chen, X., Sanders, M. E., Chakravarthy, A. B., Shyr, Y., & Pietenpol, J. A. (2011). Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. The Journal of Clinical Investigation, 121(7), 2750–2767. https://doi.org/10.1172/JCI45014

193. Teng, Y. H. F., Tan, W. J., Thike, A. A., Cheok, P. Y., Tse, G. M. K., Wong, N. S., Yip, G. W. C., Bay, B. H., & Tan, P. H. (2011). Mutations in the epidermal growth factor receptor (EGFR) gene in triple negative breast cancer: possible implications for targeted therapy. Breast Cancer Research : BCR, 13(2), R35. https://doi.org/10.1186/BCR2857

194. Park, H. S., Jang, M. H., Kim, E. J., Kim, H. J., Lee, H. J., Kim, Y. J., Kim, J. H., Kang, E., Kim, S. W., Kim, I. A., & Park, S. Y. (2014). High EGFR gene copy number predicts poor outcome in triple-negative breast cancer. Modern Pathology : An Official Journal of the United States and Canadian Academy of Pathology, Inc, 27(9), 1212–1222. https://doi.org/10.1038/MODPATHOL.2013.251

195. Nabholtz, J. M., Chalabi, N., Radosevic-Robin, N., Dauplat, M. M., Mouret-Reynier, M. A., van Praagh, I., Servent, V., Jacquin, J., Benmammar, K. E., Kullab, S., Bahadoor, M. R. K., Kwiatkowski, F., Cayre, A., Abrial, C., Durando, X., Bignon, Y. J., Chollet, P., & Penault-Llorca, F. (2016). Multicentric neoadjuvant pilot Phase II study of cetuximab combined with docetaxel in operable triple negative breast cancer. International Journal of Cancer, 138(9), 2274–2280. https://doi.org/10.1002/IJC.29952

196. Yardley, D. A., Ward, P. J., Daniel, B. R., Eakle, J. F., Lamar, R. E., Lane, C. M., & Hainsworth, J. D. (2016). Panitumumab, Gemcitabine, and Carboplatin as Treatment for Women With Metastatic Triple-Negative Breast Cancer: A Sarah Cannon Research Institute Phase II Trial. Clinical Breast Cancer, 16(5), 349–355. https://doi.org/10.1016/J.CLBC.2016.05.006

197. Finn, R. S., Press, M. F., Dering, J., Arbushites, M., Koehler, M., Oliva, C., Williams, L. S., & di Leo, A. (2009). oestrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (HER2), and epidermal growth factor receptor expression and benefit from lapatinib in a randomised trial of paclitaxel with lapatinib or placebo as first-line treatment in HER2-negative or unknown metastatic breast cancer. Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, 27(24), 3908–3915. https://doi.org/10.1200/JCO.2008.18.1925

198. Drilon, A., Laetsch, T. W., Kummar, S., DuBois, S. G., Lassen, U. N., Demetri, G. D., Nathenson, M., Doebele, R. C., Farago, A. F., Pappo, A. S., Turpin, B., Dowlati, A., Brose, M. S., Mascarenhas, L., Federman, N., Berlin, J., El-Deiry, W. S., Baik, C., Deeken, J., … Hyman, D. M. (2018). Efficacy of Larotrectinib in TRK Fusion–Positive Cancers in Adults and Children . New England Journal of Medicine, 378(8), 731–739. https://doi.org/10.1056/NEJMOA1714448/SUPPL_FILE/NEJMOA1714448_DISCLOSURES.PDF

199. Doebele, R. C., Drilon, A., Paz-Ares, L., Siena, S., Shaw, A. T., Farago, A. F., Blakely, C. M., Seto, T., Cho, B. C., Tosi, D., Besse, B., Chawla, S. P., Bazhenova, L., Krauss, J. C., Chae, Y. K., Barve, M., Garrido-Laguna, I., Liu, S. v., Conkling, P., … Demetri, G. D. (2020). Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1–2 trials. The Lancet Oncology, 21(2), 271–282. https://doi.org/10.1016/S1470-2045(19)30691-6

200. Goldenberg, D. M., Stein, R., & Sharkey, R. M. (2018). The emergence of trophoblast cell-surface antigen 2 (TROP-2) as a novel cancer target. Oncotarget, 9(48), 28989–29006. https://doi.org/10.18632/ONCOTARGET.25615

201. Bardia, A., Hurvitz, S. A., Tolaney, S. M., Loirat, D., Punie, K., Oliveira, M., Brufsky, A., Sardesai, S. D., Kalinsky, K., Zelnak, A. B., Weaver, R., Traina, T., Dalenc, F., Aftimos, P., Lynce, F., Diab, S., Cortés, J., O'Shaughnessy, J., Diéras, V., … Rugo, H. S. (2021). Sacituzumab Govitecan in Metastatic Triple-Negative Breast Cancer. New England Journal of Medicine, 384(16), 1529–1541. https://doi.org/10.1056/NEJMoa2028485

202. Harrison, H., Farnie, G., Howell, S. J., Rock, R. E., Stylianou, S., Brennan, K. R., Bundred, N. J., & Clarke, R. B. (2010). Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. Cancer Research, 70(2), 709–718. https://doi.org/10.1158/0008-5472.CAN-09-1681

203. Qiu, M., Peng, Q., Jiang, I., Carroll, C., Han, G., Rymer, I., Lippincott, J., Zachwieja, J., Gajiwala, K., Kraynov, E., Thibault, S., Stone, D., Gao, Y., Sofia, S., Gallo, J., Li, G., Yang, J., Li, K., & Wei, P. (2013). Specific inhibition of Notch1 signaling enhances the antitumour efficacy of chemotherapy in triple negative breast cancer through reduction of cancer stem cells. Cancer Letters, 328(2), 261–270. https://doi.org/10.1016/J.CANLET.2012.09.023

204. Locatelli, M. A., Aftimos, P., Claire Dees, E., LoRusso, P. M., Pegram, M. D., Awada, A., Huang, B., Cesari, R., Jiang, Y., Shaik, M. N., Kern, K. A., & Curigliano, G. (2017). Phase I study of the gamma secretase inhibitor PF-03084014 in combination with docetaxel in patients with advanced triple-negative breast cancer. Oncotarget, 8(2), 2320–2328. https://doi.org/10.18632/ONCOTARGET.13727

205. Balko, J. M., Cook, R. S., Vaught, D. B., Kuba, M. G., Miller, T. W., Bhola, N. E., Sanders, M. E., Granja-Ingram, N. M., Joshua Smith, J., Meszoely, I. M., Salter, J., Dowsett, M., Stemke-Hale, K., González-Angulo, A. M., Mills, G. B., Pinto, J. A., Gómez, H. L., & Arteaga, C. L. (2012). Profiling of residual breast cancers after neoadjuvant chemotherapy identifies DUSP4 deficiency as a mechanism of drug resistance. Nature Medicine, 18(7), 1052–1059. https://doi.org/10.1038/NM.2795

206. Pereira, C. B. L., Leal, M. F., de Souza, C. R. T., Montenegro, R. C., Rey, J. A., Carvalho, A. A., Assumpção, P. P., Khayat, A. S., Pinto, G. R., Demachki, S., de Arruda Cardoso Smith, M., & Burbano, R. R. (2013). Prognostic and Predictive Significance of MYC and KRAS Alterations in Breast Cancer from Women Treated with Neoadjuvant Chemotherapy. PLOS ONE, 8(3), e60576. https://doi.org/10.1371/JOURNAL.PONE.0060576

207. Balko, J. M., Schwarz, L. J., Bhola, N. E., Kurupi, R., Owens, P., Miller, T. W., Gómez, H., Cook, R. S., & Arteaga, C. L. (2013). Activation of MAPK Pathways due to DUSP4 Loss Promotes Cancer Stem Cell-like Phenotypes in Basal-like Breast Cancer. Cancer Research, 73(20), 6346–6358. https://doi.org/10.1158/0008-5472.CAN-13-1385

208. Brufsky, A., Kim, S. B., Zvirbule, Eniu, A., Mebis, J., Sohn, J. H., Wongchenko, M., Chohan, S., Amin, R., Yan, Y., McNally, V., Miles, D., & Loi, S. (2021). A phase II randomised trial of cobimetinib plus chemotherapy, with or without atezolizumab, as first-line treatment for patients with locally advanced or metastatic triple-negative breast cancer (COLET): primary analysis. Annals of Oncology : Official Journal of the European Society for Medical Oncology, 32(5), 652–660. https://doi.org/10.1016/J.ANNONC.2021.01.065

209. Schmid, P., Forster, M. D., Summers, Y. J., Good, J., Sarker, S.-J., Lim, L., Mousa, K., & Middleton, G. W. (2017). A study of vistusertib in combination with selumetinib in patients with advanced cancers: TORCMEK phase Ib results. Journal of Clinical Oncology, 35(15_suppl), 2548–2548. https://doi.org/10.1200/JCO.2017.35.15_suppl.2548

210. Szekely, B., Bossuyt, V., Li, X., Wali, V. B., Patwardhan, G. A., Frederick, C., Silber, A., Park, T., Harigopal, M., Pelekanou, V., Zhang, M., Yan, Q., Rimm, D. L., Bianchini, G., Hatzis, C., & Pusztai, L. (2018). Immunological differences between primary and metastatic breast cancer. Annals of Oncology, 29(11), 2232–2239. https://doi.org/10.1093/annonc/mdy399

211. Berthelet, J., Wimmer, V. C., Whitfield, H. J., Serrano, A., Boudier, T., Mangiola, S., Merdas, M., El-Saafin, F., Baloyan, D., Wilcox, J., Wilcox, S., Parslow, A. C., Papenfuss, A. T., Yeo, B., Ernst, M., Pal, B., Anderson, R. L., Davis, M. J., Rogers, K. L., … Merino, D. (2021). The site of breast cancer metastases dictates their clonal composition and reversible transcriptomic profile. Science Advances, 7(28). https://doi.org/10.1126/sciadv.abf4408

212. Trusolino, L., & Bertotti, A. (2012). Compensatory Pathways in Oncogenic Kinase Signaling and Resistance to Targeted Therapies: Six Degrees of Separation: Figure 1. Cancer Discovery, 2(10), 876–880. https://doi.org/10.1158/2159-8290.CD-12-0400

213. Bianchini, G., de Angelis, C., Licata, L., & Gianni, L. (2021). Treatment landscape of triple-negative breast cancer - expanded options, evolving needs. Nature Reviews. Clinical Oncology. https://doi.org/10.1038/S41571-021-00565-2

214. Smedley, D., Smith, K. R., Martin, A., Thomas, E. A., McDonagh, E. M., Cipriani, V., Ellingford, J. M., Arno, G., Tucci, A., Vandrovcova, J., Chan, G., Williams, H. J., Ratnaike, T., Wei, W., Stirrups, K., Ibanez, K., Moutsianas, L., Wielscher, M., Need, A., … Caulfield, M. (2021). 100,000 Genomes Pilot on Rare-Disease Diagnosis in Health Care — Preliminary Report. New England Journal of Medicine, 385(20), 1868–1880. https://doi.org/10.1056/NEJMoa2035790

215. Roepman, P., de Bruijn, E., van Lieshout, S., Schoenmaker, L., Boelens, M. C., Dubbink, H. J., Geurts-Giele, W. R. R., Groenendijk, F. H., Huibers, M. M. H., Kranendonk, M. E. G., Roemer, M. G. M., Samsom, K. G., Steehouwer, M., de Leng, W. W. J., Hoischen, A., Ylstra, B., Monkhorst, K., van der Hoeven, J. J. M., & Cuppen, E. (2021). Clinical Validation of Whole Genome Sequencing for Cancer Diagnostics. The Journal of Molecular Diagnostics, 23(7), 816–833. https://doi.org/10.1016/j.jmoldx.2021.04.011

216. Pleasance, E., Titmuss, E., Williamson, L., Kwan, H., Culibrk, L., Zhao, E. Y., Dixon, K., Fan, K., Bowlby, R., Jones, M. R., Shen, Y., Grewal, J. K., Ashkani, J., Wee, K., Grisdale, C. J., Thibodeau, M. L., Bozoky, Z., Pearson, H., Majounie, E., … Marra, M. A. (2020). Pan-cancer analysis of advanced patient tumors reveals interactions between therapy and genomic landscapes. Nature Cancer, 1(4), 452–468. https://doi.org/10.1038/s43018-020-0050-6

217. ICGC ARGO - Personalized Breast Cancer Programme - PBCP. (n.d.). Retrieved January 24, 2022, from https://www.icgc-argo.org/page/92/pbcp

218. Bruna, A., Rueda, O. M., Greenwood, W., Batra, A. S., Callari, M., Batra, R. N., Pogrebniak, K., Sandoval, J., Cassidy, J. W., Tufegdzic-Vidakovic, A., Sammut, S. J., Jones, L., Provenzano, E., Baird, R., Eirew, P., Hadfield, J., Eldridge, M., McLaren-Douglas, A., Barthorpe, A., … Caldas, C. (2016). A Biobank of Breast Cancer Explants with Preserved Intra-tumour Heterogeneity to Screen Anticancer Compounds. Cell, 167(1), 260-274.e22. https://doi.org/10.1016/J.CELL.2016.08.041/ATTACHMENT/291DBAF4-326E-4D16-BED1-31FC25EDEEF1/MMC7.XLSX

219. Woitek, R., McLean, M. A., Ursprung, S., Rueda, O. M., Garcia, R. M., Locke, M. J., Beer, L., Baxter, G., Rundo, L., Provenzano, E., Kaggie, J., Patterson, A., Frary, A., Field-Rayner, J., Papalouka, V., Kane, J., Benjamin, A. J. V., Gill, A. B., Priest, A. N., … Gallagher, F. A. (2021). Hyperpolarized Carbon-13 MRI for Early Response Assessment of Neoadjuvant Chemotherapy in Breast Cancer Patients. Cancer Research, 81(23), 6004–6017. https://doi.org/10.1158/0008-5472.CAN-21-1499

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

KP, LD, RL and JA each contributed to the design, literature review, writing, and editing of the manuscript. All authors agree to be accountable for the content of the work.

Funding

We would like to thank and acknowledge our funders Cancer Research UK (CRUK) (CRUK Cambridge Centre Grant Funding: RDZD141, RDZD192); Addenbrookes Charitable Trust (98100) and the Mark Foundation for Cancer Research (RDZD/174).

This research was also supported by the NIHR Cambridge Biomedical Research Centre (BRC-1215-20014). The views expressed are those of the authors and not necessarily those of the NIHR or the Department of Health and Social Care.

Acknowledgments

Acknowledgements: The CRUK Precision Breast Cancer Institute, Cambridge and the Cambridge Cancer Trials Centre Womens' Team; for their support for our work in Triple Negative Breast Cancer. We also acknowledge the CRUK Cambridge Centre for its core support. We would like to thank the patients, families and carers that inspire us to work on TNBC.

Figure 1.JPEG

Figure 1: *Current therapeutic strategies in early TNBC. A. Treatment spectrum B. Treatment modalities for escalation and de-escalation.*

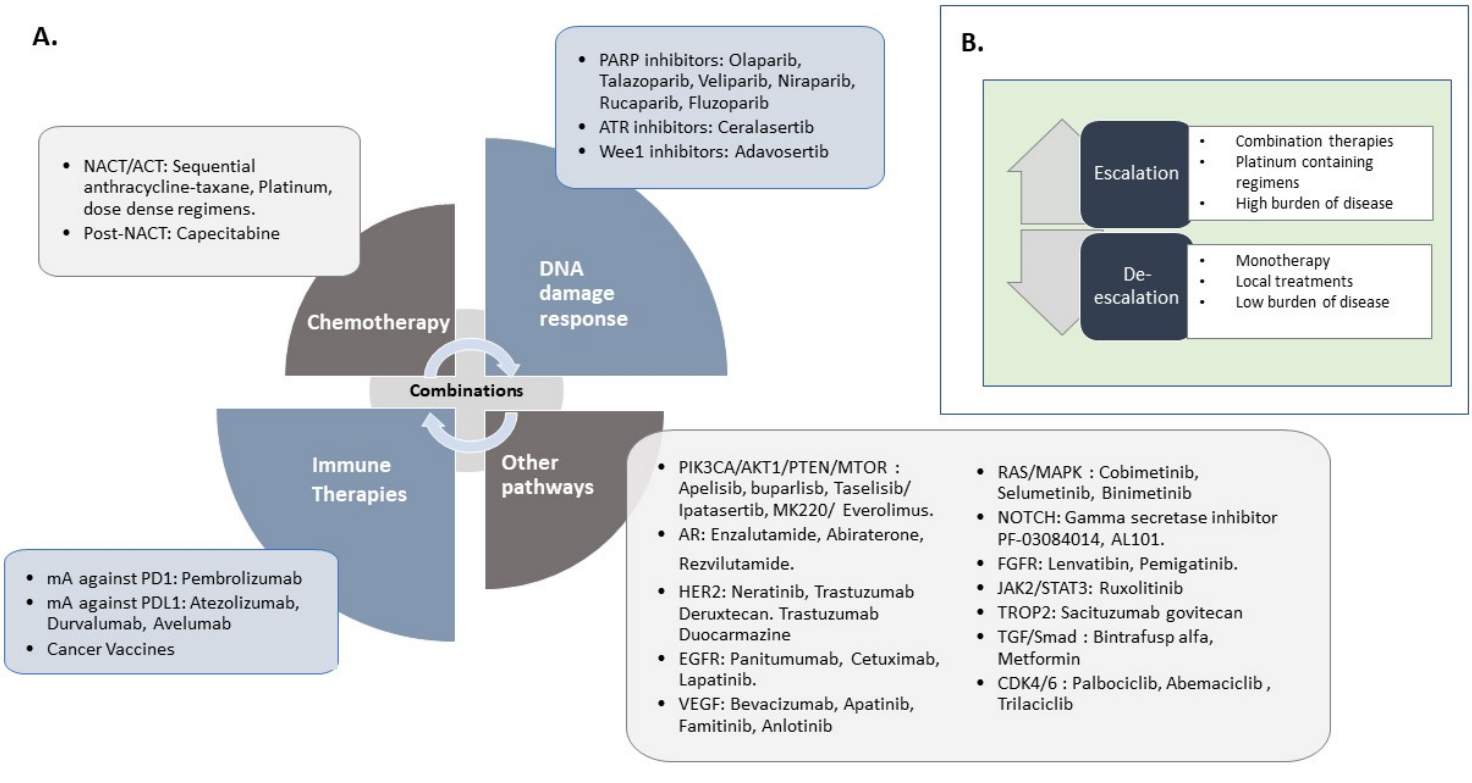

Figure 2.JPEG

Figure 2: *Biomarker landscape in TNBC.*

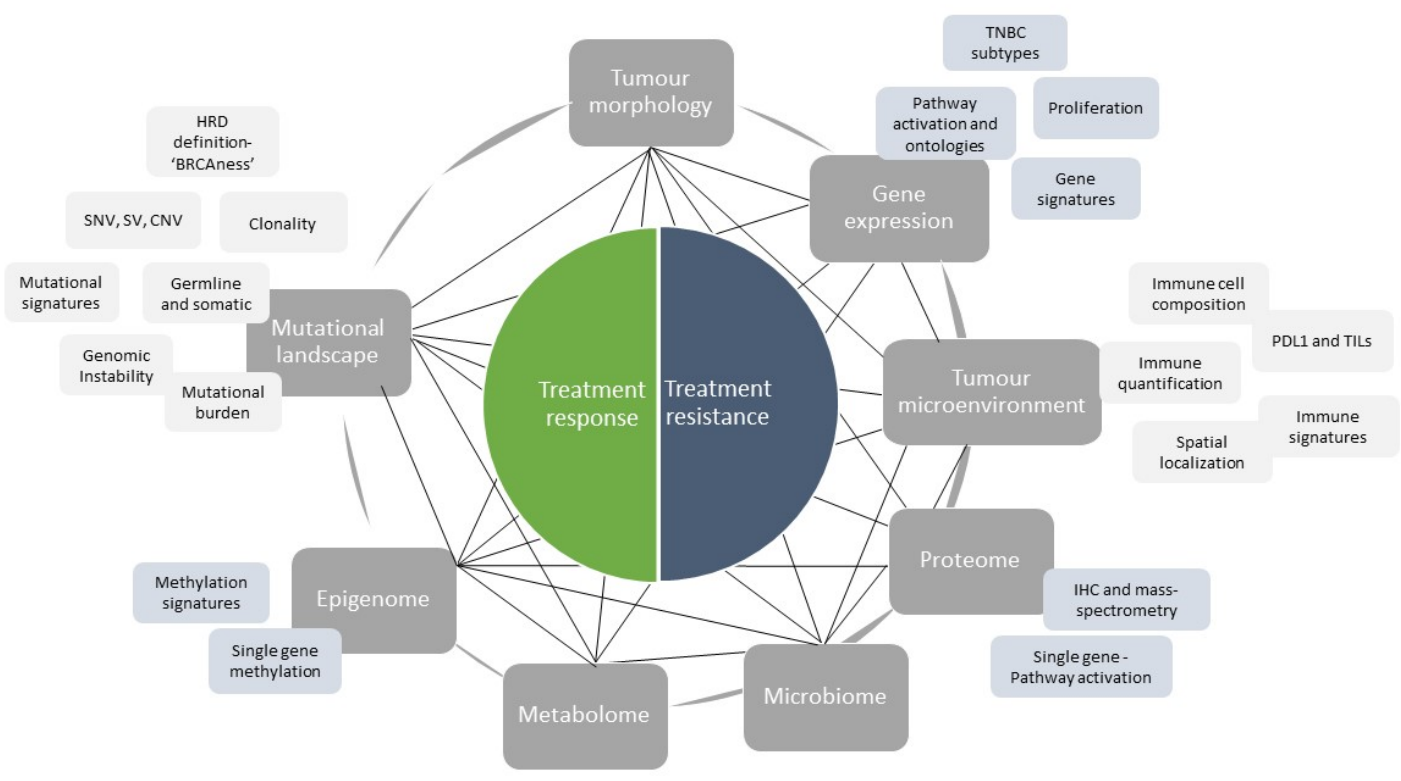

Figure 3.JPEG

Figure 3: *Proposed framework for the personalised treatment of early TNBC.*