



1 *Review*

2 **Identifying biomarkers to pair with targeting** 3 **treatments within Triple Negative Breast Cancer for** 4 **improved patient stratification**

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9 **Abstract:** The concept of precision medicine has been around for many years and recent advances
10 in high-throughput sequencing techniques are enabling this to become reality. Within the field of
11 breast cancer, a number of signatures have been developed to molecularly sub-classify tumours.
12 Notable examples recently approved by National Institute for Health and Care Excellence in the
13 UK to guide treatment decisions for ER+ HER2- patients include Prosigna® test, EndoPredict® and
14 Oncotype DX®. However, a population of still unmet need are those with Triple Negative Breast
15 Cancer (TNBC). Accounting for 15-20% of patients, this population has comparatively poor
16 prognosis and as yet no targeted treatment options. Studies have shown that some patients with
17 TNBC respond favourably to DNA damaging drugs (carboplatin) or agents which inhibit DNA
18 damage response (PARP inhibitors). Known to be a heterogeneous population, there is a need to
19 identify further TNBC patients who may benefit from these treatments. A number of signatures
20 have been identified based on association with treatment response or specific genetic
21 features/pathways however many of these were not restricted to TNBC patients and as of yet are
22 not common practice in the clinic.

23 **Keywords:** Triple negative breast cancer; targeted therapy; molecular biomarkers

24

25 **Introduction**

26 Breast cancer is the most common malignancy diagnosed in the UK, with over 55,000 new cases
27 diagnosed each year[1]. Traditionally, tumours are classified according to the presence of oestrogen
28 receptors (ER), progesterone receptors (PgR) (considered together as hormone receptor status) and
29 human epidermal growth factor receptor 2 (HER2). Treatment beyond surgery, chemotherapy and
30 radiotherapy is directed according to ER, PgR and HER2 status, with endocrine therapy or
31 trastuzumab available for patients with hormone receptor positive and HER2 positive tumours
32 respectively.

33 Accounting for approximately 10-20% of breast cancer diagnoses, triple negative breast cancers
34 (TNBC) are characterised by ER, PgR and HER2 negativity. These sub-classifications of breast cancer
35 however mask further heterogeneity and classification beyond these well-established biomarkers
36 can provide further information regarding prognosis for patients. A number of prognostic
37 algorithms are available to predict patients' risk of recurrence including Oncotype DX®,
38 MammaPrint®, EndoPredict® and Prosigna®. Many of these assays can also help to inform
39 chemotherapy decisions for patients but other than MammaPrint® are exclusively aimed at
40 hormone receptor positive patients, with the picture for TNBC being less clear. A number of
41 molecular subtypes within TNBC have been identified but as of yet there is no consensus on how
42 these should be used to inform treatment choices for patients. Given the worse prognosis for these

43 patients, there is an outstanding need to identify targeted treatment options to improve the
44 likelihood of therapeutic success in TNBC.

45 In this review we aim to summarise the current knowledge about promising targeted therapy
46 for TNBC and associated molecular signatures for treatment response.

47 Molecular heterogeneity within Triple Negative Breast Cancer

48 *Intrinsic subtypes*

49 A number of attempts have been made to sub-classify breast cancer tumours to further explain
50 the inherent heterogeneity (Table 1). One of the most renowned is the intrinsic subtypes first
51 discussed in 2000 by Perou et al[2]. Using hierarchical clustering of gene expression data from DNA
52 microarray, Perou et al identified a set of 496 genes, referred to as the “intrinsic gene subset”, which
53 showed greater between than within sample variation. Using expression patterns of the intrinsic
54 gene subset, it was shown that tumours could be classified into one of five intrinsic sub groups;
55 Basal-like, HER2-enriched, Luminal A, Luminal B and Normal like[3]. In 2009, Parker et al refined
56 the intrinsic gene subset to an optimal list of 50 genes. A final classification algorithm based on these
57 50 genes, referred to as the PAM50 classifier, was established using nearest shrunken centroid
58 methodology[4]. New samples are classified into an intrinsic subgroup based on the nearest centroid
59 method.

60 **Table 1.** Summary of breast cancer sub-classifications within TNBC.

	Subtype	Key features	Frequency in early TNBC[5-7]	Anticipated chemotherapy-sensitivity
Intrinsic subtypes	Basal-like	Gene expression similar to basal-epithelial cells. High expression of proliferation genes. High overlap with TNBC & enriched for BRCA mutations.	39-54%	High
	HER2-enriched	High expression of HER2-regulated genes. Good overlap with ER-, HER2+ tumours.	7-14%	Intermediate
	Luminal A	Gene expression similar to luminal-epithelial cells. High expression of ER-related genes.	4-5%	Low
	Luminal B	Gene expression similar to luminal-epithelial cells. Expression of ER-related genes low compared to Luminal A tumours.	4-7%	Low

	Claudin-low	High expression of epithelial-to-mesenchymal transition markers and low expression of claudins 3, 4 and 7. Lower proliferation compared to Basal-like.	25-39%	Intermediate
	Normal like	Similar expression to normal breast tissue.	1%	Low
TNBC subtypes	Basal-like 1	High expression of genes related to cell cycle, DNA damage response and proliferation.	32-36%	High
	Basal-like 2	Increased expression of growth factor signaling related genes.	18-24%	Intermediate
	Mesenchymal	Increased expression of genes related to cell motility, differentiation and growth. Absence of immune cells.	24-25%	Intermediate
	Luminal AR	Enrichment of pathways which are hormonally driven but typically hormone receptor negative. High expression of AR-related genes.	14-22%	Low
	Luminal AR	High expression of oestrogen regulated genes but typically negative by ER staining.	15-33%	Low
Baylor	Mesenchymal	High expression of genes from the following pathways: cell-cycle, mismatch repair & DNA damage.	17-28%	Intermediate
	Basal-like Immune Suppressed	Low expression of immune-related pathway genes.	29-31%	High
	Basal-like Immune Activated	High expression of immune-related pathway genes.	25-30%	High

61 The intrinsic subtypes were observed to be highly associated with ER and HER2 status with the
62 majority of triple negative tumours being classed as Basal-like[8, 9]. Despite these associations, the
63 intrinsic subtypes have been shown to be independent predictors of relapse free survival and
64 neoadjuvant chemotherapy response in untreated and treated patients respectively[4]. Given the
65 majority of TNBC patients are classified as Basal-like, tremendous efforts have been made to
66 molecularly dissect further the TNBC/non Basal-like tumours as well as to identify drug targets for
67 Basal-like tumours.

68 More recently, an additional intrinsic subtype termed Claudin-low was discovered,
69 characterised by high expression of epithelial-to-mesenchymal transition markers and low
70 expression of claudins 3, 4 and 7 [5, 10]. Gene expression profiles of Claudin-low tumours is similar
71 to that of Basal-like tumours, with a key difference being lower expression of genes associated with

72 proliferation[5]. Similar to the Basal-like subtype, Claudin-low tumours are most prevalent
73 observed in TNBC but have slightly improved prognosis, although this does not reach statistical
74 significance. Compared to the other intrinsic subtypes, response rates to anthracyclines/taxanes in
75 Claudin-low tumours is lower than that of Basal-like tumours but still higher than Luminal A and
76 Luminal B[5].

77 In 2011, Lehmann et al used cluster analysis of gene expression profiles to identify 6 genetic
78 subtypes within triple negative breast cancer; Basal-like 1 and 2, Immunomodulatory,
79 Mesenchymal, Mesenchymal Stem-like and Luminal Androgen Receptor subtypes[11]. Similar to the
80 intrinsic breast cancer subtypes, relapse free survival was significantly different between TNBC
81 subtypes ($p=0.008$) however distant metastasis free survival was not ($p=0.218$) suggesting the relapse
82 free survival difference is driven by a difference in local recurrence rates. Using TNBC cell lines,
83 Lehmann et al showed differential response rates between cell lines to different treatments.
84 However, results were not always consistent for cell-lines representing a single subtype. For
85 example, the *BRCA1* mutant cell line demonstrated a sensitivity to poly ADP ribose polymerase
86 (PARP) inhibitors which was not found for all other cell-lines representing the Basal-like subtypes.
87 They did however identify a difference in response rates to neoadjuvant taxanes in a meta-analysis
88 of 2 studies, with preferential response rates in the Basal-1 and Basal-2 subtypes. In 2013, Masuda et
89 al also showed an association between pathological complete response rates and the Lehmann
90 subtypes for 130 patients treated neo-adjuvantly with taxanes and/or anthracyclines[12]. Confirming
91 the results shown by Lehmann's group, the best response rates were seen in patients classified as
92 Basal-1[12]. These results highlight the potential to target neoadjuvant treatment with taxanes to
93 those triple negative tumours classed as Basal-like.

94 Lehmann et al further refined the 6 subtypes to 4, dropping the Immunomodulatory and
95 Mesenchymal Stem-like subtypes after identifying that these subtypes had a large number of
96 infiltrating lymphocytes or mesenchymal cells[6]. Using the refined subtypes, initially no significant
97 differences in complete response rates to neoadjuvant chemotherapy were seen (regimens contained
98 a taxane and/or anthracycline, results were consistent across regimens). A combined analysis of 4
99 datasets however showed that Basal-like 1 tumours had a significantly higher response rate
100 compared to the other subtypes. Similar results were also found in a recent study by Echavarría et
101 al[13] in which RNA sequencing data from FFPE samples was available for 94 patients treated with
102 neoadjuvant carboplatin and docetaxel. Pathological complete response rates were significantly
103 associated with the refined Lehmann subtypes ($p=0.027$) with the highest rate seen in Basal-1
104 patients with 65.6%; followed by 47.4% in Basal-2, 34.8% in Mesenchymal and 21.4% in Luminal AR
105 [13].

106 An eighty gene signature was published by Burstein et al in 2015, classifying TNBC patients
107 into 1 of 4 subtypes; Luminal-AR (LAR), Mesenchymal (MES), Basal-like Immune-Suppressed
108 (BLIS) and Basal-like Immune-Activated (BLIA) referred to as the Baylor subtypes[7]. The subtypes
109 showed significantly different disease free and disease specific survival with the worst and best
110 prognoses observed for patients classified as Basal-like Immune Suppressed and Basal-like Immune
111 Active respectively. Substantial overlap with the intrinsic subtypes was observed with the BLIS and
112 BLIA subgroups containing only Basal-like tumours whereas the LAR subgroup was a mix of
113 Luminal A, Luminal B and HER2-enriched. MES encompassed the remaining Basal-like tumours
114 and included the Normal-like samples. Some concordance with the original Lehmann TNBC 6
115 subtypes was also observed, with good overlap of the LAR subtypes according to both
116 classifications as well as the mesenchymal groups. Basal-like 1 and Basal-like 2 were both split
117 between BLIA and BLIS indicating that the signatures are picking out different features within
118 Basal-like tumours.

119 A number of studies have been carried out to provide insight regarding racial disparity
120 between subtypes. The Carolina Breast Cancer Study Phase III is a population-based study, within
121 which the PAM50 algorithm was successfully applied to 980 white or African American breast
122 cancer patients. Results showed that Basal-like tumours were more prevalent in African American
123 women compared to white women[14], this held true across age groups (<50 vs ≥ 50). On the other

124 hand, in the same study, Luminal A tumours were observed less frequently in African American
125 women[14]. Jiang et al looked at TNBC subtypes within a cohort of 360 Chinese women; compared
126 to African American and Caucasian TNBC subsets from TCGA, the Chinese cohort had a
127 significantly higher rate of Luminal AR tumours ($p < 0.05$)[15].

128 The disparities between these different breast cancer subtypes despite the generally good
129 overlap serves to highlight the complexities of the heterogeneity within TNBC. Although all three
130 subtypes provide prognostic information for patients, further work is required in order to be able to
131 personalise therapy for TNBC patients.

132 *Androgen receptor expression*

133 Androgen receptor (AR) has been shown to be expressed in 12-55% of patients with triple
134 negative breast cancer, although rates vary by study[16]. Prognosis of AR positive tumours within
135 TNBC appears conflicting; studies have shown lower chemotherapy response rates in AR expressing
136 tumours, likely due to the lower Ki67 rate in these tumours[17]. On the other hand, AR expression
137 has also been associated with overall improved prognosis, as summarised by Gerratana et al [17],
138 although chemotherapy use in the studies is not reported.

139 Although previously only considered relevant for Luminal Androgen Receptor (LAR) subtypes
140 which are largely characterised by AR expression, studies have shown that AR is also expressed in
141 non-LAR subtypes[18]. Studies in breast cancer cell lines showed reduced proliferation and
142 increased apoptosis in non-LAR lines when treated with the androgen antagonist enzalutamide,
143 even when AR expression was low[16]. A clinical study of enzalutamide in patients with
144 metastatic/locally advanced triple negative, AR positive (AR staining $>0\%$) breast cancer has also
145 reported promising results. A clinical benefit rate of 33% was observed at 16 weeks in the evaluable
146 population [19], therefore meeting the criteria for further study; other trials are ongoing.

147 TNBC tumours expressing AR have also been shown to be highly enriched for *PIK3CA* kinase
148 mutations both in cell lines [11] and patient samples[20]. Following on from this finding, Lehmann et
149 al went on to show that PI3K inhibitors combined with AR targeting had an additive effect when
150 applied in AR positive TNBC cell lines[20]. These results seem promising however pairing this
151 treatment approach with AR status is yet to be confirmed by testing within a clinical trial.

152 *Tumour Infiltrating lymphocytes*

153 A number of studies have examined the prognostic value of tumour infiltrating lymphocytes
154 (TILs) in triple negative breast cancer. Across these studies, stromal TILs have been shown to be
155 associated with outcomes in patients treated with adjuvant or neo-adjuvant chemotherapy [21-25].
156 These studies consistently showed that higher rates of stromal TILs were independently predictive
157 of improved pathological complete response, disease free survival, and overall survival regardless
158 of whether they are considered as continuous or categorised variables. Many of these studies did not
159 specifically evaluate the effect in different chemotherapy regimens, however Loi et al showed that
160 there was no significant interaction between stromal TILs and inclusion of taxanes (patients received
161 either anthracyclines or anthracyclines plus a taxane)[22]. This suggests that stromal TILs may be
162 predictive of general chemo-sensitivity in triple negative breast cancer.

163 More recently, TILs have also been looked at in early stage TNBC patients who did not receive
164 systemic therapy. A pooled analysis of 4 cohorts showed that the level of stromal TILs at diagnosis
165 was prognostic in these patients when looking at invasive disease free survival, distant disease free
166 survival and overall survival[26] with better outcomes observed in those with higher levels of TILs.
167 The study found that stromal TILs were associated with higher grade but not with other
168 clinicopathological factors, therefore the prognostic effects were shown to be independent of other
169 prognostic factors. Combined with the evidence from the earlier studies, stromal TILs look to be an
170 ideal marker for identifying patients with good prognosis regardless of whether or not systemic
171 therapy is used. Therefore, stromal TILs levels may identify a subset of patients in whom
172 chemotherapy could be avoided without compromising outcomes.

173 Promising targeted therapy for TNBC

174 *PARP inhibitors*

175 PARP inhibitors have been studied as an approach to cancer treatment for several years. As
176 summarised by Plummer, the first PARP inhibitor was given as a chemo-potentiator in combination
177 with chemotherapeutic agents in 2003[27]. Since then, increased understanding of the mechanisms
178 of action of PARP inhibitors and the different forms of DNA repair, has led to the approach of using
179 them as a single agent in patients with deficient homologous recombination repair pathways.

180 The rationale behind their use is the concept of synthetic lethality. PARP1 and PARP2 enzymes
181 are involved in the DNA repair of single strand breaks. By impairing PARP1 and 2 via use of an
182 inhibitor, the accumulation of single strand breaks can lead to double strand breaks. In the absence
183 of functioning homologous recombination repair, such as in the presence of a *BRCA1/2* mutation,
184 these double strand breaks cannot be fixed efficiently which results in cell death[28].

185 Since their first use in the early 2000s, PARP inhibitors have more recently been shown to be an
186 effective maintenance therapy in women with newly diagnosed advanced ovarian cancer with a
187 germline or somatic *BRCA1/2* mutation[29]. They have also been shown to be effective in women
188 with HER2 negative advanced/metastatic breast cancer with an inherited *BRCA1/2* mutation [30, 31]
189 and have recently been approved by the US Food and Drug Administration (FDA) for use in this
190 setting. Recent interim results from the PROfound study suggest these effects also hold true in
191 prostate cancer patients with alterations in a number of homologous recombination repair genes
192 beyond *BRCA1/2*[32]. This was a randomised phase III trial comparing the PARP inhibitor olaparib
193 with physician's choice of enzalutamide or abiraterone in men with metastatic castrate-resistant
194 prostate cancer with an alteration in one of 15 genes involved in homologous recombination repair.
195 An impressive hazard ratio of 0.49 (0.38 to 0.63) in favour of olaparib was seen for the primary
196 endpoint of radiographic progression free survival [32].

197 *Platinum agents*

198 Platinum agents such as carboplatin and cisplatin are used in cancer treatment due to their
199 ability to cause DNA double stranded breaks through the formation of DNA inter-strand
200 cross-links[33, 34]. Several phase II and III studies have shown that the addition of platinum agents
201 in the neoadjuvant setting can improve response rates in women with triple negative breast cancer
202 [35-38]. The BrighTNess and GeparSixto studies went on to look at response rates according to
203 germline BRCA mutation status and found no significant interactions between BRCA mutation
204 status and treatment group [36, 38]. Further to this, although no significant interaction was detected,
205 a difference in response rates was observed in GeparSixto but this was in fact driven by improved
206 response rates in the BRCA wildtype patients, with patients with a BRCA mutation achieving good
207 response rates regardless of the treatment group assigned. In the advanced setting however, the
208 TNT trial showed the opposite, with no benefit of carboplatin over docetaxel in the overall triple
209 negative breast cancer population but a significantly improved response rate for carboplatin
210 compared to docetaxel when analysis was restricted to those with a germline *BRCA1/2* mutation
211 [39]. These contradictory results suggest that further exploration of the biology driving tumour
212 response is required in order to identify the group of patients most likely to derive benefit from
213 platinum-based chemotherapy.

214 PARP inhibitors and platinum agents have to date largely been focussed on patients with a
215 *BRCA1/2* mutation. It is however hypothesised that a larger group of patients without *BRCA1/2*
216 mutations but with other homologous recombination repair deficiencies could also benefit from
217 these treatment approaches. Several groups are working on molecular biomarkers to identify these
218 patients as outlined later in this review.

219 *CDK4/6 inhibitors*

220 Cyclin-dependent kinase (CDK) 4/6 inhibitors work by interrupting the cell-cycle to reduce
 221 proliferation of cancer cells. To date, three CDK4/6 inhibitors (palbociclib, ribociclib and
 222 abemaciclib) have been approved by the FDA for use in patients with advanced/metastatic
 223 oestrogen positive, HER2 negative breast cancer following a number of successful trials in this
 224 disease setting [40]. Previously, triple negative breast cancers were not thought to be a good
 225 candidate for treatment with CDK4/6 inhibitors due to approximately 20% of these tumours lacking
 226 functional Retinoblastoma-like protein (Rb) [41]. Pre-clinical data however has indicated the
 227 potential for sensitive subtypes of TNBC; in particular, a study by Asghar et al showed that the LAR
 228 subtype of TNBC was CDK4/6 inhibitor sensitive in vitro and in vivo [42]. Other TNBC tumours
 229 with high RB expression, androgen receptor positivity or associated clinical characteristics are also
 230 considered potential candidates [43] and some pre-clinical research suggests a benefit of
 231 combination treatment including CDK4/6 inhibition [41]. A number of phase I or II studies of
 232 CDK4/6 inhibitors are ongoing within subsets of TNBC patients and results are awaited.

233 *Immunotherapy*

234 This year, the FDA gave approval for the combination of atezolizumab (a PD-L1 targeting
 235 immunotherapy drug) with chemotherapy in triple negative breast cancer. The approval came
 236 following the phase III IMpassion130 trial which showed an improvement in progression free
 237 survival following the addition of atezolizumab to neoadjuvant nab-paclitaxel in untreated
 238 metastatic TNBC, with a hazard ratio of 0.80 (95% confidence interval: 0.69 to 0.92)[44]. When
 239 restricted to the subgroup of patients with PD-L1 positivity, the benefit of adding atezolizumab was
 240 observed to be even more pronounced with a hazard ratio 0.62 (95% confidence interval: 0.49 to
 241 0.78). Interim analysis of overall survival did not show a statistically significant difference between
 242 treatment groups overall, but Kaplan Meier analysis suggested a longer median overall survival in
 243 those with PD-L1 positive tumours.

244 Interim results of the Keynote173 trial were also presented last year. These showed that high
 245 stromal TILs and PD-L1 were associated with improved pathological complete response and
 246 objective response rates in primary TNBC which had been treated with the immunotherapy
 247 pembrolizumab and neoadjuvant chemotherapy[45]. No comparison was made to a regimen
 248 excluding the immunotherapy, but combined with the results from IMpassion130 suggest that
 249 immunotherapies in TNBC could be effective in patients with PD-L1 positive tumours. The benefit
 250 of immunotherapies in patients without this marker however is more uncertain at present.

251 High mutational burden has also been suggested as a potential indicator of immunotherapy
 252 sensitivity. Recent results from one cohort of the TAPUR study showed 37% disease control rate in
 253 patients with metastatic breast cancer with high tumour mutational burden treated with
 254 pembrolizumab[46]. Further evidence however is required to support the ability of this potential
 255 biomarker to direct treatment.

256 A number of other immunotherapy trials in TNBC are ongoing which will provide further
 257 insight, however many of these are in unselected patients (Table 2) and there is still a need for
 258 identification of robust biomarkers to predict benefit of immunotherapy.

259 **Table 2.** Selection of ongoing trials of pembrolizumab or atezolizumab in TNBC (source:
 260 ClinicalTrials.gov).

Setting	ClinicalTrials.gov identifier	Study name	Treatment	Planned/ final sample size	Status
Adjuvant	NCT03036488	KEYNOTE-522	Pembrolizumab + chemotherapy vs placebo + chemotherapy	1174	Open no longer recruiting

	NCT02954874	Pembrolizumab in Treating Patients with Triple Negative Breast Cancer	Pembrolizumab vs. observation	1000	Recruiting
	NCT03498716	IMpassion030	Atezolizumab + chemotherapy vs. chemotherapy	2300	Recruiting
	NCT02620280	NeoTRIPaPDL1	Atezolizumab + chemotherapy vs. chemotherapy	278	Open no longer recruiting
	NCT03639948	NeoPACT	Pembrolizumab + chemotherapy	100	Recruiting
Neoadjuvant	NCT03281954	Clinical Trial of Neoadjuvant Chemotherapy With Atezolizumab or Placebo in Patients With Triple-Negative Breast Cancer Followed After Surgery by Atezolizumab or Placebo	Atezolizumab vs. placebo	1520	Recruiting
	NCT02530489	Nab-Paclitaxel and Atezolizumab Before Surgery in Treating Patients With Triple Negative Breast Cancer	Atezolizumab + chemotherapy	37	Recruiting
	NCT02819518	KEYNOTE-355	Pembrolizumab + chemotherapy vs placebo + chemotherapy	882	Open no longer recruiting
	NCT03121352	Carboplatin, Nab-Paclitaxel and Pembrolizumab for Metastatic Triple-Negative Breast Cancer	Pembrolizumab + chemotherapy	30	Open no longer recruiting
Metastatic/ locally advanced	NCT02555657	KEYNOTE-119	Pembrolizumab vs. chemotherapy	622	Open no longer recruiting
	NCT02447003	KEYNOTE-086	Pembrolizumab	285	Open no longer recruiting
	NCT03125902	IMpassion131	Atezolizumab + chemotherapy vs. placebo + chemotherapy	600	Recruiting
	NCT03371017	IMpassion132	Atezolizumab vs. placebo	350	Recruiting

	NCT02734290	Standard of Care Chemotherapy Plus Pembrolizumab for Breast Cancer	Pembrolizumab + chemotherapy	88	Recruiting
	NCT03206203	Carboplatin With or Without Atezolizumab in Treating Patients With Stage IV Triple Negative Breast Cancer	Atezolizumab + chemotherapy vs. chemotherapy	185	Recruiting

261 Identification of molecular signatures for treatment response

262 *Homologous recombination deficiency (HRD)*

263 Beyond *BRCA1/2* mutations, wider homologous recombination deficiency subgroups have been
 264 defined to identify a broader subgroup of patients who may benefit from specific treatment
 265 strategies. Loss of heterozygosity (LOH), telomeric allelic imbalance (TAI) and large-scale state
 266 transitions (LST) are independent measures of genomic instability each associated with *BRCA*
 267 mutational status[47-49]. Timms et al showed that a combined score generated by taking the mean of
 268 the scores was better at identifying samples with homologous recombination deficiency than the
 269 individual scores [50], this is referred to as the HRD score. Within triple negative breast cancer
 270 patients, an association between HRD score or HR deficiency (defined as HRD score ≥ 42 or a
 271 *BRCA1/2* mutation) and pathological complete response to platinum agents has been observed [51].
 272 However, a similar association between HR deficiency and response was also observed with
 273 anthracycline or taxane based neoadjuvant chemotherapy in a separate retrospective study [52].
 274 Similar results were observed in the advanced setting [39] suggesting the HRD score is a prognostic
 275 marker within triple negative breast cancer patients and not predictive of response to a particular
 276 treatment.

277 More recently developed, HRDetect is a mutational signature model developed using lasso
 278 logistic regression to identify patients with homologous recombination deficiency [53]. Developed to
 279 identify patients with a *BRCA* deficiency the model has 98.7% sensitivity and was able to identify a
 280 number of patients with deficiencies which had not previously been picked up, classifying a larger
 281 cohort of patients who could benefit from *BRCA*/homologous recombination deficient targeted
 282 treatment strategies.

283 Earlier this year, Staaf et al published the results of applying the HRDetect signature to TNBC
 284 patients from the observational SCAN-B study in Sweden[54]. Of the 237 patients with evaluable
 285 samples, they found that 58.6% of TNBC patients had high HRDetect scores (defined as a score >0.7).
 286 HRDetect high tumours were enriched for Basal-like (PAM50 Basal-like and TNBCtype Basal-like 1)
 287 and Mesenchymal tumours. On the other hand, HRDetect low tumours were enriched for Luminal
 288 AR tumours and had more PAM50 non-Basal-like (mainly HER2 enriched and normal-like) tumours
 289 compared to the high tumours. Of the patients treated with standard of care adjuvant chemotherapy
 290 (regimens varied but fluorouracil, epirubicin and cyclophosphamide \pm taxane was common), those
 291 with high HRDetect were shown to have better outcomes as assessed by invasive disease free
 292 survival. This led the authors to conclude HRDetect high tumours to be more chemo-sensitive than
 293 patients with low HRDetect scores[54].

294 Further to this, when calculated in samples from the personalized oncogenomics project,
 295 another observational study, the model was shown to be associated with improved outcomes in
 296 advanced breast cancer patients treated with platinum agents [55]. It should however be noted that
 297 the sample size in this study was small and further analysis in a larger prospective study is required
 298 to confirm these results.
 299

300 *Mutational signature*

301 Substantial work has been carried out to characterise mutational signatures by whole genome
302 and/or exome sequencing in cancer which reflect the different mutations which have occurred
303 within a tumour. One particular mutational signature, referred to as signature 3, has been shown to
304 be highly associated with the presence of *BRCA1* and *BRCA2* mutations[56, 57] in breast and other
305 tumour types. It was noted however that a number of cases without *BRCA1* or *BRCA2* mutations
306 also exhibited high levels of signature 3. This led Polak et al to explore the association of signature 3
307 with the wider homologous recombination repair pathway. They identified associations of the
308 signature with epi-genetic silencing of *BRCA1* and mutation/methylation in other key genes from
309 the homologous recombination pathway including *PALB2* and *RAD51C*[58].

310 Mutational signature 3 was used in the development of the HRDetect signature however to our
311 knowledge has not been tested alone for prognostic or predictive ability to date. Consequently, there
312 is little evidence regarding prognosis or predictive ability of this signature within TNBC.

313 *Gene expression signatures*

314 Several gene expression signatures related to DNA damage response have also been developed
315 in an attempt to identify sub-populations of patients likely to derive benefit from therapeutic
316 approaches. A number of methodologies have been employed based on association of gene
317 expression data with either biological features related to DNA damage response or DNA damaging
318 treatment sensitivity.

319 Two promising signatures for treatment response that have come out of these approaches are
320 the PARPi7 and BRCA1ness signatures. The first was published in 2012 by Daemen et al[59] who
321 identified a subset of genes for which transcriptional levels were associated with sensitivity to the
322 PARP inhibitor olaparib across a number of breast cancer cell lines. From an initial list of 118
323 candidate genes taken from different DNA repair pathways, 7 were taken forward into signature
324 development and combined using the weighted voting algorithm to define the PARPi7 signature.
325 When applied to unselected breast cancer patients who had not been treated with a PARP inhibitor,
326 8-21% of patients were predicted to be PARP inhibitor sensitive based on the signature, identifying a
327 substantial proportion of patients who may benefit from this treatment approach. Based on
328 biological features rather than treatment sensitivity, the BRCA1like signature was developed to
329 identify patients classed as BRCA1-like according to DNA copy number profiles[60]. Using diagonal
330 linear discriminant analysis, 77 genes were identified which could classify samples between the
331 BRCA1-like and non-BRCA1-like groups. In order to create a signature more utilisable in the clinic,
332 the authors adapted the signature to be centroid based and a threshold was selected to give a high
333 sensitivity of 96.7% and specificity of 73.1% in classifying patients.

334 These two signatures were subsequently applied to the 72 patients randomised to veliparib and
335 carboplatin arm and the 44 HER2-negative controls within the I-SPY 2 breast cancer trial. The
336 interaction between each biomarker and treatment group was statistically significant even after
337 adjustment for hormone receptor status (p-values of 0.001 and 0.02 for PARPi7 and BRCA1ness
338 respectively)[61]. This supports the notion of these two signatures being predictive of PARP
339 inhibition sensitivity although the authors acknowledge that veliparib and carboplatin were given in
340 combination so it cannot be determined whether the signatures are predicting sensitivity to the
341 combination or one of the individual agents. Results also require validation in a larger dataset as
342 sample size for these subgroup analyses was small.

343 Given the known association between the Fanconi anaemia/BRCA pathway with DNA damage
344 repair deficiencies, Mulligan et al sought to develop a DNA damage repair deficiency (DDR) assay
345 based on the molecular characterisation of patients with Fanconi anaemia[62]. Using Affymetrix
346 microarray they identified differentially expressed probesets between patients with Fanconi
347 anaemia and a set of patient controls. A number of breast cancer samples (n = 107) enriched for
348 BRCA mutations were split into separate ER positive and negative datasets. Within each dataset,
349 hierarchical clustering was applied and clusters representing the molecular processes associated
350 with Fanconi anaemia were classed as DDR positive, with remaining samples classified DDR

351 negative. The classified ER positive and ER negative datasets were then re-combined and a 44-gene
352 expression signature was identified to accurately classify samples as DDRD positive or negative. The
353 authors went on to show that the signature could predict response to fluorouracil, Adriamycin and
354 cyclophosphamide (FAC) chemotherapy in the neo-adjuvant setting and fluorouracil,
355 epirubicin and cyclophosphamide (FEC) in the adjuvant. The signature could not however predict
356 survival outcomes in an independent cohort of patients who did not receive cytotoxic
357 chemotherapy. These results suggest the potential use of this signature to predict which patients
358 may benefit from the addition of anthracycline based chemotherapy.

359 The DDRD signature was subsequently successfully applied to 381 early TNBC patients treated
360 with an adjuvant anthracycline containing regimen from the SWOG 9313 study. The signature was
361 shown to be predictive of disease-free survival and overall survival, with high scores associated with
362 improved outcomes independent of other prognostic factors[63]. The study also looked at stromal
363 TILS density and found a positive correlation between this and the DDRD signature suggesting the
364 potential for DDRD high tumours to be targeted with immune checkpoint inhibitors[63].

365 Adopting a slightly different approach based on chromosomal instability, Carter et al correlated
366 10,151 genes with total functional aneuploidy across a number of pan-cancer datasets to develop the
367 CIN70 signature[64]. A chromosomal instability score was calculated for each gene by summing the
368 correlation rank of the gene across the datasets. The CIN70 signature is then composed of the top 70
369 genes with the highest CIN score; a simpler version was also created using the top 25 genes only
370 (CIN25). The authors showed that the CIN signatures could be used to predict clinical outcome
371 across a number of datasets including breast cancer patients and furthermore provided additional
372 prognostic information above tumour grade alone. The CIN70 signature was also explored within
373 the I-SPY2 trial where no significant interaction between the signature and treatment group was
374 observed ($p=0.22$ after adjustment for hormone receptor status)[61]. It therefore remains to be seen if
375 high chromosomal instability, as determined by this signature, is targetable or simply prognostic
376 across treatments as treatment specific data was not available in the original paper.
377

378 *Promise of Liquid biopsies in clinical management*

379 One emerging biomarker for prognosis is the evaluation of circulating tumour DNA (ctDNA).
380 This is a non-invasive assessment method based on the detection of ctDNA which has been released
381 from the tumour into the blood stream. Garcia-Murillas et al looked at the use of ctDNA measured in
382 blood at a single post-operative timepoint or from serial sampling to predict outcomes in early breast
383 cancer unselected for hormone receptor status[65]. Presence of ctDNA within both the single time
384 point and serial sampling could predict relapse across tumour types including within TNBC patients
385 ($p=0.009$ and 0.003)[65]. Sample size was small with just 11 and 13 TNBC patients with available
386 samples for single time-point and serial sampling respectively, the results however are supported by
387 other small studies restricted to TNBC patients with similar findings [66, 67] suggesting the potential
388 for ctDNA as a biomarker for relapse. What is currently less clear is whether ctDNA detection can be
389 used to direct treatment. One trial trying to provide insights for this this is the cTRACK-TN trial
390 (NCT03145961). Patients are followed up with serial ctDNA screening after completion of primary
391 treatment, with randomization between pembrolizumab and observation in those with ctDNA
392 detected prior to 12 months.

393 **Conclusion**

394 Over the last 20 years, increased availability and improvements in molecular profiling has
395 uncovered the vast molecular heterogeneity present within TNBC. Molecular subtypes based on
396 gene expression profiles have been identified and shown to confer vastly different risk profiles
397 which may help inform decisions regarding chemotherapy use.

398 Standard treatment approaches in patients with TNBC was previously limited to surgery with
399 chemotherapy and/or radiotherapy, with a distinct lack of available targeted therapies. Treatment

400 pathways however are now evolving with the recent approval of PARP inhibitors for patients with a
401 *BRCA1/2* mutation and ongoing research into the use of CDK4/6 inhibitors and immunotherapies.

402 A number of signatures predicting treatment response have also been developed for TNBC
403 patients with some showing promising results in retrospective analyses. Many of these however still
404 require validation within prospective trials in order to be brought forward into the clinic. With the
405 advent of multi-omics technologies, more advanced computational approaches are being applied to
406 integrate such high-dimensional biological data with patient outcomes to derive robust genomic
407 signatures to inform better clinical management and next generation clinical trial designs.

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