

Imaging Biomarkers for Precision Medicine in Locally Advanced Breast Cancer

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Published version

TRAN, W T, CHILDS, Charmaine, PROBST, Heidi, FARHAT, G and CZARNOTA, G J (2018). Imaging Biomarkers for Precision Medicine in Locally Advanced Breast Cancer. *Journal of Medical Imaging and Radiation Sciences*, 49 (4), 342-351.

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3 **Commentary: Imaging Biomarkers for Precision Medicine in**
4 **Locally Advanced Breast Cancer**
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21 **ACCEPTED FOR PUBLICATION TO JOURNAL OF MEDICAL IMAGING AND RADIATION**
22 **SCIENCES: SEPTEMBER 18 2017**
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31 *Keywords: Imaging Biomarkers, Chemotherapy response, Locally advanced breast cancer*
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46 **ABSTRACT**

47

48 Guidelines from the American National Comprehensive Cancer Network (NCCN)
49 recommend neoadjuvant chemotherapy (NAC) to patients with locally advanced breast
50 cancer (LABC) to downstage tumors before surgery. However, only a small fraction
51 (15-17%) of LABC patients achieve complete pathologic response (pCR), i.e. no
52 residual tumor in the breast, after treatment. Measuring tumor response during
53 neoadjuvant chemotherapy can potentially help physicians adapt treatment thus,
54 potentially improving the pCR rate.

55 Recently, imaging biomarkers that are used to measure the tumor's functional
56 and biological features have been studied as pre-treatment markers for pCR or as an
57 indicator for intra-treatment tumor response. Also, imaging biomarkers have been the
58 focus of intense research to characterize tumor heterogeneity as well as to advance our
59 understanding of the principle mechanisms behind chemoresistance. Advances in
60 investigational radiology are moving rapidly to high-resolution imaging, capturing
61 metabolic data, performing tissue characterization and statistical modelling of imaging
62 biomarkers, with an endpoint of personalized medicine in breast cancer treatment. In
63 this commentary, we present studies within the framework of imaging biomarkers used
64 to measure breast tumor response to chemotherapy. Current studies are showing that
65 significant progress has been made in the accuracy of measuring tumor response either
66 before or during chemotherapy, yet the challenges at the forefront of these works
67 include translational gaps such as needing large-scale clinical trials for validation, and
68 standardization of imaging methods. However, the ongoing research is showing that
69 imaging biomarkers may play an important role in personalized treatments for LABC.

INTRODUCTION AND BACKGROUND

70
71

72 Recent guidelines by the National Comprehensive Cancer Network (NCCN)
73 define locally advanced breast cancer (LABC) as stage 3 breast cancer [1]. Thus, large
74 tumors greater than 5 cm with regional lymph node involvement or inoperable breast
75 cancer, defined as having skin and/or chest wall involvement are locally advanced [1,
76 2]. Incidence rates of LABC in the United States accounted for 12.4% of new breast
77 cancer cases in 2015 and 8.5% of cases in the United Kingdom [3, 4]. Survival data
78 from the *SEER* registry (Statistics, Epidemiology, and End-Results Program) in the
79 United States have indicated poor survival outcomes [5, 6]; mortality rates were 52%
80 for stage 3A breast cancer and 48% for stage 3B disease [5]. Similarly, data from the
81 United Kingdom showed that between 2002-2006, only 55.1% of women with stage 3
82 breast cancer survived beyond 5-years (recent data unavailable) [7]. Poor survival
83 outcomes are caused by factors associated with genetics, tumor heterogeneity,
84 vascularity, oxygenation and some intrinsic molecular features such as estrogen
85 receptor (ER) and human epidermal growth factor receptor-2 (Her2) expression.

86 The recommended treatment course for LABC is neoadjuvant chemotherapy
87 (NAC), followed by surgery, then radiation [1, 8]. Studies emerged in the 1970s
88 demonstrating the benefit of pre-operative chemotherapy to downstage tumors before
89 surgery, since reducing the tumor size and extent can make surgical excision possible
90 [9]. The additional benefit of using NAC includes enabling lumpectomy rather than
91 total mastectomy, if for example there are clinical indications (tumor size and margins,
92 nodal status and patient preference after NAC) [1, 9-12]. Neoadjuvant chemotherapy is
93 also desirable since monitoring tumor response during therapy would allow potentially

94 adapting therapies based on clinical response [13, 14]. It has been shown that
95 pathological complete response (pCR), defined as having no residual tumor after NAC
96 can serve as a prognostic indicator for survival and is supported by work from the
97 German Breast Group (GBG) who reported improved disease-free survival for luminal
98 B/Her2-, Her2+ (non-luminal), and triple negative (ER-/PR-/Her2-) breast cancers that
99 achieve pCR [15]. Furthermore, a meta-analysis of 3,182 locally advanced breast
100 cancer patients demonstrated improved survival in patients who achieved pCR after
101 neoadjuvant chemotherapy (overall survival=2.3-7.6 years) [16]. In another study, 87%
102 of pCR patients survived beyond 5 years, in comparison to patients who demonstrated
103 partial or no response [17]. The results of these studies suggest that pathology
104 endpoints after neoadjuvant chemotherapy can provide vital information on survival
105 outcomes and thus, pCR is in part, the desired clinical outcome for administering NAC.
106 However, despite the significant improvements in treatment strategies over past
107 decades, only a small fraction of patients will achieve pCR. Previous studies have
108 reported pCR rates of only 15.2%-17.4% following neoadjuvant chemotherapy [16, 18].
109 With less than a quarter of treated patients achieving a complete pathological response,
110 new ways of improving outcome and survival for patients with LABC are a real clinical
111 challenge for the future.

112 To address these challenges, there has been research interests in exploring new
113 ways to assess intra-treatment responses to NAC as well in finding ways to predict the
114 treatment response even before the use of chemotherapy; in other words, to make a
115 prognosis for the presumed efficacy of the treatment. A deeper understanding of tumor
116 behavior and customizing treatments based on genetic, patient and other biological

117 information are referred to as precision medicine. The tailoring of treatments is also
118 termed personalized medicine.

119 To help achieve this, a greater understanding is needed of tumor biology; the
120 way the tumor influences for example, angiogenesis, drives cell proliferation and
121 ultimately how the tumor cells die from chemotherapy are important considerations for
122 precision medicine in oncology. In this commentary, we present past and current
123 studies focusing on imaging biomarkers in breast cancer.

124

125 **HALLMARKS OF CHEMORESISTANCE AND** 126 **CHEMOEFFICACY**

127

128

129 *Intertumor and Intratumor Heterogeneity Contributes to Chemoresistance*

130

131 Intertumor heterogeneity is, in part, caused by intrinsic variances in molecular
132 features such as estrogen receptor (ER), progesterone receptor (PR) and human
133 epidermal growth factor-2 receptor (Her2). Data from 50,571 women in the United
134 States indicated that 72.7% of women exhibit luminal A-like breast cancer; while 12.2%
135 express basal-like breast cancers. A smaller portion of patients exhibit luminal B-like
136 breast cancer (10.3%); whereas only 4.6% of all breast cancer patients have Her2
137 overexpressed (Her2+) breast cancer. [19]. These differences in tumor profiles can
138 require different targeted therapies, such as Trastuzumab in the case of Her2
139 overexpressed tumors. Breast cancer subtypes also demonstrate variable responses to
140 neoadjuvant chemotherapy [15, 20, 21]. Reports from over 6,000 patients have
141 indicated that basal-type, and HER2+ breast cancers have the highest rate of pCR to

142 anthracycline- and taxane-based chemotherapies. In contrast, luminal A and luminal B
143 breast cancers (i.e. ER+, PR+) are highly resistant to chemotherapy [15]. Rodent
144 models have demonstrated that luminal breast cancer cells exhibit stem-cell-like
145 behaviors that are genetically driven for tumor cell immortality, higher rates of
146 differentiation, and rapid proliferation [22]. Some studies have also suggested that
147 basal-type tumors have dysfunctional cell-repair mechanisms in comparison to luminal
148 A and luminal B tumors that make it more susceptible to chemotherapy-induced DNA
149 damage [23].

150 Intratumor heterogeneity is another treatment resistance challenge. It is
151 characterized as a mixture of cells and stromal features that constitute tumor
152 composition. Tumors are also constructed from a variety of other cell-types such as
153 fibroblasts, immune cells, adipocytes and normal breast epithelial cells [24, 25]. The
154 complexity of intratumor heterogeneity is confounded by morphological differences such
155 as enlarged or shrunken cell sizes from tumor cell proliferation and cycling. These
156 events also cause substructural alterations that result in condensed nuclear bodies and
157 organelle reorganization [26]. Taken together, tumors are composed of disorganized
158 and aberrant cells, and circulating biomolecules that are “woven” into a turbulent
159 vascular scaffold and environment. Other physiological conditions that lead to
160 intratumor heterogeneity include fluctuating interstitial fluid, variable vascular perfusion
161 and circulating biomolecules [27]. These aberrations inhibit effective delivery of
162 chemotherapies and, thus, result in variable treatment response. Taken together, the
163 heterogeneous and tortuous tumor matrix is a significant treatment challenge in breast
164 cancer [28].

165

166 *Mechanisms of Chemoefficacy*

167

168 One mechanism by which chemotherapy agents exert their therapeutic effect is
169 by committing tumor cells to apoptosis [29, 30]. In comparison to other forms of cell
170 death, such as necrosis, apoptotic cell death is energy dependent, genetically controlled
171 and morphologically distinct (i.e., developing apoptotic bodies, cell shrinking and
172 nuclear condensation) (**Figure 1**) [31]. Apoptosis has been identified in primary breast
173 tumors treated with neoadjuvant chemotherapy *in situ*. Studies by Chang et al. (2000)
174 and Ellis et al. (1997) demonstrated that there was an increase in apoptosis in
175 responsive tumors and detected as early as 24 hours after the administration of
176 chemotherapy [32, 33]. Chang et al. (2000) showed that increased apoptosis was
177 linked to complete pathologic response where there was no residual or palpable
178 disease after therapy [32]. Buchholz et al. (2003) also measured the apoptotic activity
179 in breast tumors after 48 hours of chemotherapy. Patients who had a 25% increase in
180 the apoptotic activity had gone on to achieve pCR. The apoptotic activity was
181 significantly different to patients who did not achieve pCR ($P < 0.015$) [34]. Although
182 only a small number of clinical studies have examined serial breast tumor biopsies to
183 measure apoptosis *in situ*, the findings to date have indicated agreement with
184 laboratory-based experiments for other tumor types *in vitro* [35-37].

185 Alterations in the tumor's vascular organization are also important hallmarks of
186 chemoefficacy. An important property of malignancies is the abnormal vascular
187 architecture, which contributes to a spatially heterogeneous environment [38]. The

188 vascular morphology and layout have been well studied; blood vessels are
189 disorganized, distributed unevenly, immature and leaky, which also affects the tumor's
190 response to treatment [39]. The tortuous vessel formations have been shown
191 previously to inhibit drug efficacy by secreting cell-protective factors against
192 chemotherapy insult [40, 41] . Additionally, abnormal morphologies such as variable
193 vessel diameters and weak junctions in the vessel walls have been demonstrated to
194 inhibit efficacious drug delivery since leaky vessels mitigate drug concentrations in
195 tumors for effective therapeutic effect [42, 43]. Additionally, the uneven vascular
196 scaffold creates areas with variable and high interstitial fluid pressure, which resists the
197 transport of cytotoxic agents into the stroma [28, 41, 44]. Solid tumors that respond to
198 chemotherapy exhibit characteristic patterns in their vessel reorganization [38]. Jain et
199 al. (2005) described these patterns as vascular “normalization” by which the vascular
200 architecture is reconfigured to eliminate inefficient, saccular, leaky and immature vessel
201 formations (**Figure 2**) [38]. This results in improved oxygen delivery and cytotoxic
202 efficacy. In highly responsive tumors, the vasculature eventually regresses and limits
203 the nutrient supply to tumor cells [45]. The net effect is a regression in the vascular
204 density in tumors. Consequently, this leads to spatial and structural changes in the
205 tumor.

206 Taken together, the important characteristics of tumor response to chemotherapy
207 include vascular normalization and regression, cell death and changes in the tissue
208 composition. These characteristics are the focus of detection using imaging biomarkers.
209

210 **IMAGING BIOMARKERS AS INDICATORS FOR**
211 **CHEMORESPONSE**
212

213 *Conventional Imaging Methods*

214 Conventional imaging from magnetic resonance imaging (MRI), computed
215 tomography (CT) and B-mode ultrasound (US) are used to measure tumor size changes
216 during NAC. Radiological response criteria are graded using RECIST 1.1 (*Response*
217 *Criteria in Solid Tumors*) guidelines [46]. However, major limitations for measuring
218 tumor size changes include: 1) dependency on user expertise to identify the lesion; 2)
219 distinguishing tumor boundaries on multiple scan planes in the case of MRI and CT; 3)
220 a change in the tumor's size may take several weeks before it is detectable, which limits
221 early detection and; 4) size measurements may be conflated with fibrosis, collagen, fatty
222 tissue and inflammation in the breast.

223 Quantitative imaging biomarkers addresses the limitations associated with
224 conventional imaging. Quantitative imaging biomarker techniques measure the
225 biological and functional tumor features previously outlined such as cell metabolism, cell
226 death and vascular reorganization. The overall purpose of investigating imaging
227 biomarkers in oncological studies is to achieve optimal accuracy of imaging biomarker
228 features with pathology endpoints such as pCR. Recent imaging methods are
229 described below and biomarker measurements are outlined in **Table 1**.

230 *Magnetic Resonance Imaging Biomarkers*

231 MRI-based imaging biomarkers can be extracted from diffusion-weighted imaging
232 (DWI-MRI), dynamic contrast enhanced imaging (DCE-MRI), blood-oxygen level
233 dependent imaging (BOLD-MRI) and MRI-spectroscopy (MRI-SPEC). These

234 techniques are capable of mapping tumor oxygenation, vascularization, metabolism and
235 the extracellular matrix as response markers to neoadjuvant chemotherapy in breast
236 cancer (**Table 1**). Diffusion-weighted MR measures the diffusion of water molecules
237 (i.e. Brownian motion) in tissue [47, 48]. Tissue contrast can be displayed in DW-MRI
238 imaging based on areas of high and low water diffusion; where areas of low water
239 motion (i.e. tumors) demonstrate an enhanced signal. Previous studies have
240 demonstrated that areas with low water motion are associated with malignant tissue due
241 to densely arranged cells which limit the motion of water in the extracellular space [48].
242 Extrinsic contrast imaging techniques include dynamic contrast enhanced imaging
243 (DCE-MRI) which detects the concentration of an injected contrast agent (gadolinium
244 chelate) in the intravascular and extravascular space using primarily T1-weighted
245 signals [47]. DCE-MRI images provide information on tumor vascularity and blood flow
246 and measure the gadolinium “wash-in” and “wash-out”. Tumors preferentially
247 accumulate gadolinium from an increased vascular supply compared to normal tissue,
248 and therefore demonstrate an enhanced signal in MRI [49]. Blood-oxygen level
249 dependent (BOLD-MRI) imaging is also used to measure the tumor vascularity, and
250 tumor oxygenation. This is accomplished by detecting deoxyhemoglobin, which is
251 paramagnetic and therefore results in signal loss in T2-weighted images [50].

252

253 *Positron-Emission Tomography (PET)*

254 PET imaging monitors metabolic activity by tracking the cellular uptake of a
255 glucose analogue, [¹⁸F]-fluorodeoxyglucose (FDG). FDG is injected intravenously,
256 transported into cells like glucose, and is labelled with a radioactive tracer that

257 demonstrates radioactive decay, permitting PET imaging to map metabolic activity in
258 tissue. Increased FDG-uptake (standard uptake value, SUV) has been demonstrated in
259 tumors since tumor metabolism is greater compared to normal tissue. PET imaging can,
260 therefore, serve to identify the extent of malignancies [51]. PET imaging is achieved
261 with the release of a gamma-ray photon that is detected by a photon-detection device
262 during radioactive decay, known as positron-electron annihilation. Another radiotracer
263 used in PET is the radionuclide $^{15}\text{O-H}_2\text{O}$, which is used to measure tumor blood flow;
264 where the distribution of water can be equated to blood activity in blood vessels [52].
265 Previous work from Duch et al. (2009) showed that the intratreatment change in SUV
266 (ΔSUV , 2 cycles of chemotherapy) differentiated between pathologic response groups
267 (responders vs. non-responders) with a sensitivity of 77% and specificity of 80%, using
268 a cut-off value of 40% [53].

269

270 *Diffuse Optical Spectroscopy (DOS)*

271 Diffuse optical spectroscopy (DOS) imaging can measure tumor response to
272 chemotherapy by focusing on changes in tissue composition [54-56]. Maps of tumor
273 physiological features, such as hemoglobin, are computed from tissue-optical properties
274 that are based on near-infrared optical scattering and absorption within the near-infrared
275 spectrum (600-1100 nm) [57]. For breast tissue, significant optical absorbers include
276 oxy-hemoglobin (HbO_2), deoxy-hemoglobin (Hb), water (H_2O) and lipids (Li) [57].
277 Chromophore concentrations can be estimated by measuring the absorption co-efficient
278 $[\mu_a]$ and using Beer's law equation [58]. Also, tissue optical parameters such as the
279 reduced scattering co-efficient $[\mu'_s]$ can provide additional information on tissue

280 microstructure ($\sim 0.2 \mu\text{m}$); corresponding to optical scattering effects from mitochondria
281 and the cell nucleus [57, 59]. Other DOS parameters, such as the scatter power and
282 scatter amplitude, calculated by using the power-law function, are representative of the
283 tissue's substructure, which is related to cellularity, cell arrangement, and light-scatterer
284 spatial distributions [60]. As a result, DOS imaging can demonstrate a good sensitivity
285 to the biochemical characteristics of breast tumors that undergo changes from
286 neoadjuvant chemotherapy. Previous work by Cerussi *et al.* (2011) indicated that
287 hemoglobin-based parameters demonstrated significant differences between pCR vs.
288 non-pCR patients ($p < 0.05$) [58]. Early indicators of treatment response were reported
289 by Robyler *et al.* (2011) and showed an "oxy-hemoglobin flare" in responders after one
290 week of treatment [54]. In another study by Ueda *et al.* (2012), the baseline oxygen
291 saturation demonstrated significant differences between pCR and non-pCR patients
292 ($p < 0.01$), and corresponded to a sensitivity and specificity of 75.0% and 73.3%,
293 respectively [61].

294

295 *Ultrasound Imaging Biomarkers*

296 Ultrasound imaging biomarkers are obtained by mechanical imaging such as
297 elastography (which is considered semi-quantitative), or functional imaging such as
298 power-Doppler ultrasound and quantitative ultrasound spectroscopy (QUS). Ultrasound
299 elastography measures tissue stiffness, which characterizes tissue biomechanical
300 properties. Tumors are "stiffer" than the surrounding normal parenchyma because they
301 are comprised of densely populated and rapidly dividing cells, as well as increased
302 vasculature and fibroglandular components that alter its mechanical properties [62-64].

303 Tissue stiffness can be measured in terms of tissue stress and strain using shear-wave
304 elastography or compression-based elastography. Evans et al. (2013) reported that
305 stiffer tumors were significantly correlated to a higher residual cancer burden index
306 (RCBI), which indicates poor pathologic response at the end of chemotherapy (Pearson
307 correlation coefficient=0.23, $P<0.004$) [65].

308 Functional US-based imaging techniques include power Doppler imaging that
309 assess tumor vasculature from the frequency shift and amplitude (power) of the
310 ultrasound backscatter signal from scatterers in the blood vessels [66]. An emerging
311 field includes quantitative ultrasound spectroscopy, which uses the spectral information
312 of the ultrasound radiofrequency (RF) signals to characterize morphological changes in
313 tumor cells associated with apoptosis caused by chemotherapy [37, 67]. To date, QUS
314 has been used to measure intratreatment response; showing significant changes in the
315 spectral parameters for chemoresponding patients as early as one week after treatment
316 initiation [68]. Also, recent results have demonstrated that pre-treatment QUS
317 parameters can predict NAC response in patients with an accuracy of 88%; while
318 demonstrating a high correlation to survival outcomes [69].

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Technique	Biomarker Measurements	Treatment Points Studied	Ref.
Magnetic Resonance Imaging			
DWI-MRI	<ul style="list-style-type: none"> • Extracellular water motion • Tumor-cell density • Tissue micro-structure • Cell membrane integrity • Cell membrane permeability 	<ul style="list-style-type: none"> • Pre-treatment • Intratreatment • Post-chemotherapy 	[48]
DCE-MRI	<ul style="list-style-type: none"> • Vascular permeability • Dynamic blood flow 		[47]
BOLD	<ul style="list-style-type: none"> • Tumor oxygenation • Tumor vascularity • Angiogenesis • Blood Volume • Blood Flow 		[47]
SPECT	<ul style="list-style-type: none"> • Reduction in mitotic count • Tumor cellularity • Cell membrane integrity • Tumor metabolism • Tissue composition (lipid) 		[70]
Positron-Emission Tomography			
¹⁸ F-FDG	<ul style="list-style-type: none"> • Tumor metabolism 	<ul style="list-style-type: none"> • Pre-treatment • Intratreatment • Post-chemotherapy 	[71]
¹⁵ O-H ₂ O	<ul style="list-style-type: none"> • Tumor blood flow 		[50]
Diffuse Optical Spectroscopy			
DOS	<ul style="list-style-type: none"> • Metabolism • Cell activity • Vascular Density • Edema • Breast tissue composition • Cellularity • Cell death and Morphology • Tissue contrast • Hypoxia 	<ul style="list-style-type: none"> • Pre-treatment • Intratreatment • Post-chemotherapy 	[52]
			[57]
			[58]
			[77]
Ultrasound			
Elastography	<ul style="list-style-type: none"> • Tumor progression • Extracellular matrix • Collagen crosslinking • Tissue composition (fibrosis) 	<ul style="list-style-type: none"> • Pre-treatment • Intratreatment • Post-chemotherapy 	[64]
Power Doppler	<ul style="list-style-type: none"> • Vascular blood flow • Blood perfusion • Vascularity 		[62]
QUS	<ul style="list-style-type: none"> • Tumor Cell Death (Apoptosis) • Cell Morphology and Distribution 		[65]
			[78]
			[79]
			[80]

326 **Table 1.** Imaging biomarker studies have included MRI, PET imaging, DOS, and
327 ultrasound based imaging. The studies have included response assessment using
328 various biological features at various stages of chemotherapy treatment: before
329 treatment (pre-treatment), intratreatment, and post-treatment

IMAGE TEXTURE ANALYSIS AND MACHINE LEARNING

Other imaging biomarker features can be extracted from image-texture analysis.

Texture analysis refers to mathematical methods that can apply second-order statistical methods to yield texture features of an image. Feature-extraction methods, such as those based on grey-level co-occurrence matrices (GLCM), can be applied to compute the probabilities of relative pixel intensities of images from the spatial distribution of their voxels [81]. This is useful for quantifying image heterogeneities and their application has extended to discriminating benign vs. malignant breast lesions in breast radiographs [82]. Texture analysis has also been useful in X-ray mammography [83], MRI [84, 85], positron-emission tomography (PET) [86], and ultrasound [87] to identify malignant lesions and for discriminating and characterizing various tissue types [88]. In other breast studies, GLCM analysis has been under investigation for utility to classify benign and malignant lesions using planar (2D) and volumetric (3D) MRI images [84, 89]. Additionally, GLCM analysis has been used to segment lesion borders of stellate (malignant) breast masses [90].

For therapy evaluation, texture analysis has also been used to discriminate breast tumor response to NAC from various imaging modalities [82, 91, 92]. Texture features of the image carry important information about the tumor's properties, corresponding to heterogeneity within the tumor itself [90]. Such techniques have been applied with computer-aided, machine-learning techniques for statistical modelling [93]. Machine learning classification algorithms include support vector machines (SVM), k-nearest neighbor (k-NN), naïve Bayes, and artificial neural networks (ANN) that can be used to classify response groups by pattern recognition and spatial probabilities within a

355 feature space. These methods have recently been applied to quantitative ultrasound
356 (QUS) imaging and have demonstrated high classification accuracy in responders and
357 non-responders at early phases of NAC treatment [68]. These previous findings
358 suggested that textural features can provide information on the microstructural
359 biological characteristics carried in the parametric layout, not otherwise detected using
360 the mean parametric measurements [68].

361

362 **STATUS OF IMAGING BIOMARKERS FOR PERSONALIZED** 363 **MEDICINE IN BREAST CANCER**

364

365 Adopting imaging biomarkers as a decision-making tool in the clinic involves
366 several steps that originate with laboratory investigations and, following the translational
367 research pathway progress to clinical trials. Here, it is pertinent to discuss the current
368 demand from patients and clinicians for imaging biomarkers in the clinic, the
369 translational obstacles and how generalizable imaging biomarker models are for
370 measuring breast cancer response to NAC. The demand for imaging biomarkers has
371 been highlighted recently by a UK-based working group that identified critical research
372 gaps and translational priorities for breast cancer. Their report highlighted the
373 importance of exploiting both biospecimen-based markers and imaging for guiding
374 breast cancer treatment. Below are the major considerations outlined by their group
375 [94]:

376

- 377 1. Selection of therapies should be offered on an individual basis and using level-
378 one evidence. Personalized treatments are the best approach. Important

- 379 considerations include optimizing the treatment time-course from individual
380 tumor and patient data. Currently, overtreatment is a clinical challenge.
- 381 2. An assessment of the tumor's underlying biology is essential. Tumor metrics
382 may help assess the patient's metastatic risk and predict drug resistance. The
383 tumor's behaviors from its cellular characteristics, molecular features,
384 angiogenic pathways and stromal conditions (i.e. hypoxia, altered metabolism)
385 may aid in understanding the impact on therapeutic interventions. This may be
386 achieved by using functional and metabolic medical imaging modalities.
 - 387 3. Clinical decision-making tools will be integral in the management and treatment
388 of breast cancer patients. For example, imaging biomarkers could be used to
389 predict prognosis and response to chemotherapy. Imaging modalities will
390 permit potentially non-invasive, serial measurements that monitor the dynamic
391 tumor changes over time.
 - 392 4. High risk populations include triple negative breast cancer patients and
393 research needs to address prognostic and predictive biomarkers for this patient
394 population. In general, tumor heterogeneity is a treatment challenge and
395 stratification of patients is needed in future studies for better treatment
396 strategies.
 - 397 5. Both clinical and financial effectiveness should be considered while
398 implementing new decision-making tools for clinical use.

399
400 The need for biomarkers in medicine has been identified for decades. In the early
401 2000s, the human genome project was completed to identify and map out thousands of

402 genes in human cells [95, 96]. Since then, great efforts have been made in cataloguing
403 and identifying gene signatures involved in disease progression, drug metabolism and
404 treatment resistance across several disorders like cardiovascular disease, infectious
405 diseases and cancer [97]. A major focus in genomic oncology has been to identify
406 predictors for chemotherapy-resistance in breast cancer [97, 98]. Indeed, thousands of
407 gene markers have been studied as predictors to therapy response in cancer. Yet, one
408 of the most notable works include the validation of a 21-gene assay (Oncotype-DX) that
409 predicts the probability that patients would benefit from adjuvant chemotherapy. The
410 assay includes genes that have been shown to potentiate higher prognostic risk factors
411 [98]. The 21-gene signatures have undergone validation in over 10,000 patients. The
412 NSABP study B-14 trial demonstrated that Oncotype DX was shown to predict
413 recurrence in patients treated with Tamoxifen [99]; while a parallel study (NSABP study
414 B-20) showed the benefit of the assay for predicting chemotherapy response [100].
415 The benefits from Oncotype DX biomarker testing are recognized as useful for a subset
416 of breast patients; namely, in hormone-receptor-positive, Her2-negative, axillary node-
417 negative breast cancer [101, 102]. The Oncotype-DX assay is one example of how
418 specimen-derived biomarker discoveries have been adopted by clinicians to guide
419 treatment and enhance personalized medicine. It also demonstrates the several
420 validation hurdles that biomarker studies undergo before clinical acceptance and that
421 biomarkers themselves may not be generalizable for all breast cancer subtypes. In
422 comparison to imaging biomarkers, no such imaging biomarkers have reached the
423 clinical adoption stage comparable to biospecimen biomarkers to guide treatment
424 decisions like Oncotype DX for breast cancer.

425 Despite the significant efforts to investigate imaging biomarkers for clinical use,
426 many of the identified biomarkers have not surpassed initial research hypothesis
427 testing; thus, never having reached large-scale clinical trials for robust clinical validation.
428 In fact, emerging research that could potentially guide treatments often falls through two
429 major translational gaps [103]. These gaps were previously outlined by Cancer
430 Research UK (CRUK) and the European Organization for Research and Treatment of
431 Cancer (EORTC) working group; specifically: 1) validation of the biomarkers through
432 initial scientific testing (i.e. are the imaging biomarkers robustly tested and capable of
433 answering the scientific or medical hypothesis?) and; 2) validation of the imaging
434 biomarkers as a clinical-decision tool (i.e. have the imaging biomarkers undergone the
435 appropriate clinical trial to be used and generalized for patients?). Integrating and using
436 imaging biomarkers in practice necessitates marker validation, generalizability and cost-
437 benefit analysis [94, 103]. To date, imaging biomarkers have surpassed the first
438 translational gap to address scientific hypothesis testing, but have yet to succeed in the
439 subsequent clinical research testing stage for robust validation. Major limitations
440 include repeatability and reproducibility of results and the standardization of assessing
441 tumor response, i.e., imaging parameters and protocols, time intervals and establishing
442 test cut-off points.

443 Taken together, imaging biomarkers are proving to have great potential for use in
444 locally advanced breast cancer treatment. The limitations for routine clinical use
445 involves the need for multicenter trials for validation and improvements on study design
446 and laying out a standard imaging protocol. To address these, this will involve
447 determining the optimal imaging time-points to assess intratreatment response and

448 establishing the appropriate test cut-off points that classify patients into the responder
449 vs. non-responder category. The aim, nevertheless, is to develop imaging biomarkers to
450 permit response-predictive or response-adaptive therapy to move away from a one-size
451 fits all approach towards personalized cancer care.

452

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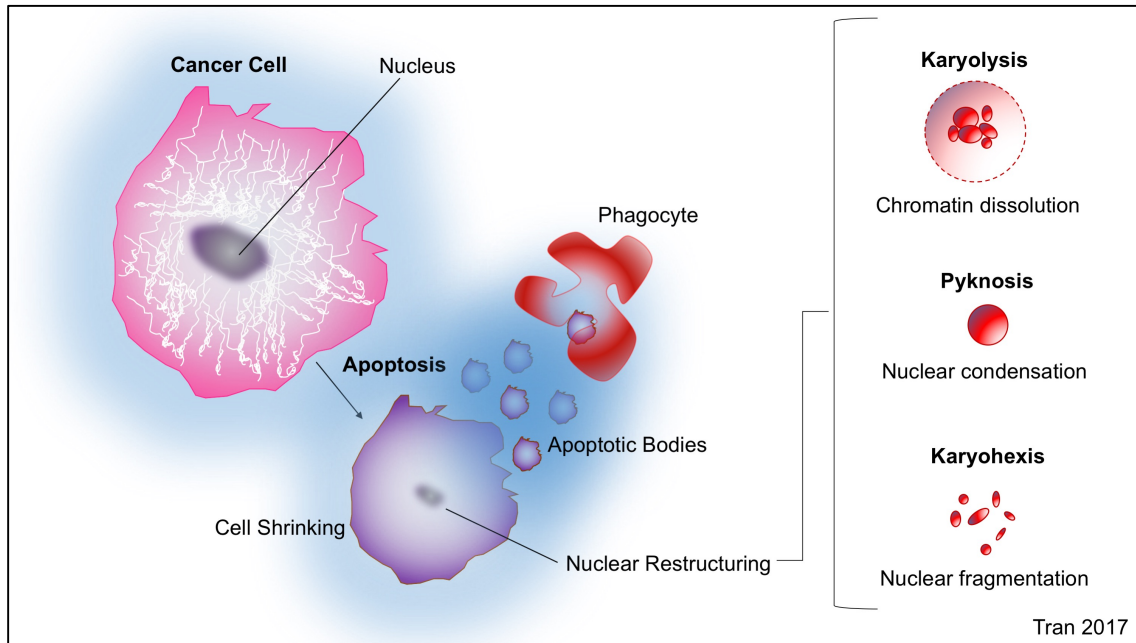
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716 **Figure 1**
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720 **Figure 1: Apoptosis in cancer cells.** Apoptosis is characterized as an energy
721 dependent mechanism where cells undergo programmed morphological changes.
722 Chemotherapies induce apoptosis in tumor cells and this results in cell shrinking and
723 nuclear restructuring such as karyolysis, pyknosis and karyorrhexis.
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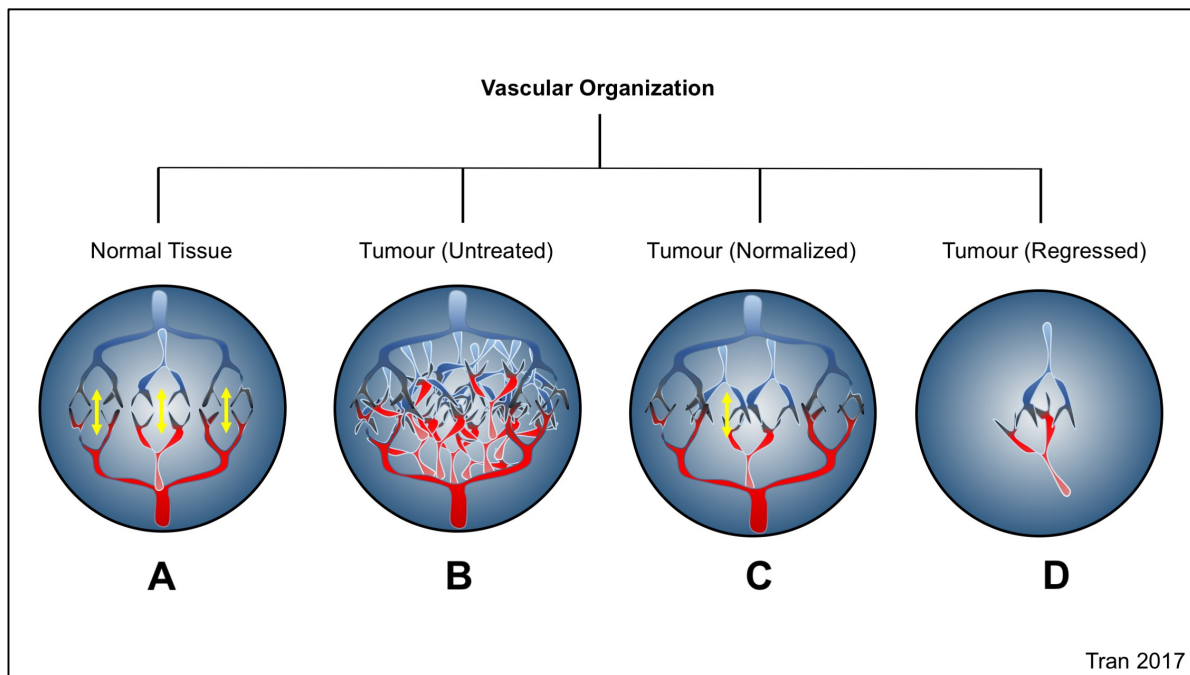
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735 **Figure 2**

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738 **Figure 2:** A comparison of the vascular organization. **A.** Normal tissue exhibits well-
739 organized vasculature, which permit exchange of biomolecules and gas (arrows). **B.**
740 Untreated tumors show high density vasculature and do not permit free exchange of
741 biomolecules and gasses. **C.** Normalized tumors demonstrate greater organization
742 closer to that of normal tissue. **D.** In regressed tumors, the vasculature may be absent,
743 or minimal. (Figure adapted from Jain et al., 2005 [45]).

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