

## 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, [<sup>18</sup>F]-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 ( $\Delta\text{SUV}$ , 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 ( $\text{HbO}_2$ ), deoxy-hemoglobin (Hb), water ( $\text{H}_2\text{O}$ ) 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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325

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

## 330 **IMAGE TEXTURE ANALYSIS AND MACHINE LEARNING**

331  
332  
333

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

334 Texture analysis refers to mathematical methods that can apply second-order statistical  
335 methods to yield texture features of an image. Feature-extraction methods, such as  
336 those based on grey-level co-occurrence matrices (GLCM), can be applied to compute  
337 the probabilities of relative pixel intensities of images from the spatial distribution of their  
338 voxels [81]. This is useful for quantifying image heterogeneities and their application  
339 has extended to discriminating benign vs. malignant breast lesions in breast  
340 radiographs [82]. Texture analysis has also been useful in X-ray mammography [83],  
341 MRI [84, 85], positron-emission tomography (PET) [86], and ultrasound [87] to identify  
342 malignant lesions and for discriminating and characterizing various tissue types [88]. In  
343 other breast studies, GLCM analysis has been under investigation for utility to classify  
344 benign and malignant lesions using planar (2D) and volumetric (3D) MRI images [84,  
345 89]. Additionally, GLCM analysis has been used to segment lesion borders of stellate  
346 (malignant) breast masses [90].

347 For therapy evaluation, texture analysis has also been used to discriminate  
348 breast tumor response to NAC from various imaging modalities [82, 91, 92]. Texture  
349 features of the image carry important information about the tumor's properties,  
350 corresponding to heterogeneity within the tumor itself [90]. Such techniques have been  
351 applied with computer-aided, machine-learning techniques for statistical modelling [93].  
352 Machine learning classification algorithms include support vector machines (SVM), k-  
353 nearest neighbor (k-NN), naïve Bayes, and artificial neural networks (ANN) that can be  
354 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

## 453 REFERENCES

454

- 455 1. National Comprehensive Cancer Network. *Breast Cancer (Version 2.2016)*. 2016  
456 [cited 2017 January 17, 2017]; Available from:  
457 [https://www.nccn.org/professionals/physician\\_gls/f\\_guidelines.asp](https://www.nccn.org/professionals/physician_gls/f_guidelines.asp).
- 458 2. Garg, P.K. and G. Prakash, *Current definition of locally advanced breast cancer*.  
459 *Curr Oncol*, 2015. **22**(5): p. e409-10.
- 460 3. Iqbal, J., et al., *Differences in breast cancer stage at diagnosis and cancer-*  
461 *specific survival by race and ethnicity in the United States*. *JAMA*, 2015. **313**(2):  
462 p. 165-73.
- 463 4. Cancer Research UK. *Proportion of Cancers Diagnosed at Each Stage, All Ages,*  
464 *England*. 2016 2016 [cited 2016 January 17 2017]; Available from:  
465 [http://www.cancerresearchuk.org/sites/default/files/cstream-](http://www.cancerresearchuk.org/sites/default/files/cstream-node/inc_stage_breast_0.pdf)  
466 [node/inc\\_stage\\_breast\\_0.pdf](http://www.cancerresearchuk.org/sites/default/files/cstream-node/inc_stage_breast_0.pdf).
- 467 5. Newman, L.A., *Epidemiology of locally advanced breast cancer*. *Semin Radiat*  
468 *Oncol*, 2009. **19**(4): p. 195-203.
- 469 6. Tryfonidis, K., et al., *Management of locally advanced breast cancer-*  
470 *perspectives and future directions*. *Nat Rev Clin Oncol*, 2015. **12**(3): p. 147-62.
- 471 7. Cancer Research UK. *Breast Cancer (C50): 2002-2006; Five-Year Relative*  
472 *Survival (%) by Stage, Adults Aged 15-99, Former Anglia Cancer Network*. 2016  
473 2016 [cited 2016 January 17 2017]; Available from:  
474 [http://www.cancerresearchuk.org/sites/default/files/cstream-](http://www.cancerresearchuk.org/sites/default/files/cstream-node/surv_5yr_stage_w_breast_0.pdf)  
475 [node/surv\\_5yr\\_stage\\_w\\_breast\\_0.pdf](http://www.cancerresearchuk.org/sites/default/files/cstream-node/surv_5yr_stage_w_breast_0.pdf).
- 476 8. Lee, M.C. and L.A. Newman, *Management of patients with locally advanced*  
477 *breast cancer*. *Surg Clin North Am*, 2007. **87**(2): p. 379-98, ix.
- 478 9. Alvarado-Cabrero, I., et al., *Incidence of pathologic complete response in women*  
479 *treated with preoperative chemotherapy for locally advanced breast cancer:*  
480 *correlation of histology, hormone receptor status, Her2/Neu, and gross*  
481 *pathologic findings*. *Ann Diagn Pathol*, 2009. **13**(3): p. 151-7.
- 482 10. Broadwater, J.R., et al., *Mastectomy following preoperative chemotherapy. Strict*  
483 *operative criteria control operative morbidity*. *Ann Surg*, 1991. **213**(2): p. 126-9.
- 484 11. Chia, S., et al., *Locally advanced and inflammatory breast cancer*. *J Clin Oncol*,  
485 2008. **26**(5): p. 786-90.
- 486 12. Chen, A.M., et al., *Breast conservation after neoadjuvant chemotherapy*. *Cancer*,  
487 2005. **103**(4): p. 689-95.

- 488 13. Cance, W.G., et al., *Long-term outcome of neoadjuvant therapy for locally*  
489 *advanced breast carcinoma: effective clinical downstaging allows breast*  
490 *preservation and predicts outstanding local control and survival.* Ann Surg, 2002.  
491 **236**(3): p. 295-302; discussion 302-3.
- 492 14. Honig, A., et al., *State of the art of neoadjuvant chemotherapy in breast cancer:*  
493 *rationale, results and recent developments.* Ger Med Sci, 2005. **3**: p. Doc08.
- 494 15. von Minckwitz, G., et al., *Definition and impact of pathologic complete response*  
495 *on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer*  
496 *subtypes.* J Clin Oncol, 2012. **30**(15): p. 1796-804.
- 497 16. Kong, X., et al., *Meta-analysis confirms achieving pathological complete*  
498 *response after neoadjuvant chemotherapy predicts favourable prognosis for*  
499 *breast cancer patients.* Eur J Cancer, 2011. **47**(14): p. 2084-90.
- 500 17. Kuerer, H.M., et al., *Clinical course of breast cancer patients with complete*  
501 *pathologic primary tumor and axillary lymph node response to doxorubicin-based*  
502 *neoadjuvant chemotherapy.* J Clin Oncol, 1999. **17**(2): p. 460-9.
- 503 18. Chollet, P., et al., *Prognostic significance of a complete pathological response*  
504 *after induction chemotherapy in operable breast cancer.* Br J Cancer, 2002.  
505 **86**(7): p. 1041-6.
- 506 19. Howlander, N., et al., *US incidence of breast cancer subtypes defined by joint*  
507 *hormone receptor and HER2 status.* J Natl Cancer Inst, 2014. **106**(5).
- 508 20. Carey, L.A., et al., *The triple negative paradox: primary tumor chemosensitivity of*  
509 *breast cancer subtypes.* Clin Cancer Res, 2007. **13**(8): p. 2329-34.
- 510 21. Rouzier, R., et al., *Breast cancer molecular subtypes respond differently to*  
511 *preoperative chemotherapy.* Clin Cancer Res, 2005. **11**(16): p. 5678-85.
- 512 22. Sims, A.H., et al., *Origins of breast cancer subtypes and therapeutic implications.*  
513 Nat Clin Pract Oncol, 2007. **4**(9): p. 516-25.
- 514 23. Desmedt, C., et al., *Biological processes associated with breast cancer clinical*  
515 *outcome depend on the molecular subtypes.* Clin Cancer Res, 2008. **14**(16): p.  
516 5158-65.
- 517 24. Polyak, K., *Heterogeneity in breast cancer.* J Clin Invest, 2011. **121**(10): p. 3786-  
518 8.
- 519 25. Pietras, K. and A. Ostman, *Hallmarks of cancer: interactions with the tumor*  
520 *stroma.* Exp Cell Res, 2010. **316**(8): p. 1324-31.
- 521 26. Swanton, C., *Intratumor heterogeneity: evolution through space and time.* Cancer  
522 Res, 2012. **72**(19): p. 4875-82.
- 523 27. O'Connor, J.P., et al., *Imaging intratumor heterogeneity: role in therapy*  
524 *response, resistance, and clinical outcome.* Clin Cancer Res, 2015. **21**(2): p.  
525 249-57.
- 526 28. Rofstad, E.K., K. Galappathi, and B.S. Mathiesen, *Tumor interstitial fluid*  
527 *pressure-a link between tumor hypoxia, microvascular density, and lymph node*  
528 *metastasis.* Neoplasia, 2014. **16**(7): p. 586-94.
- 529 29. Bold, R.J., P.M. Termuhlen, and D.J. McConkey, *Apoptosis, cancer and cancer*  
530 *therapy.* Surg Oncol, 1997. **6**(3): p. 133-42.
- 531 30. Mizutani, H., et al., *Mechanism of apoptosis induced by doxorubicin through the*  
532 *generation of hydrogen peroxide.* Life Sci, 2005. **76**(13): p. 1439-53.

- 533 31. Majno, G. and I. Joris, *Apoptosis, oncosis, and necrosis. An overview of cell*  
534 *death*. Am J Pathol, 1995. **146**(1): p. 3-15.
- 535 32. Chang, J., et al., *Apoptosis and Proliferation as Predictors of Chemotherapy*  
536 *Response in Patients with Breast Carcinoma*. Cancer, 2000. **89**(11): p. 2145-  
537 2152.
- 538 33. Ellis, P.A., et al., *Preoperative chemotherapy induces apoptosis in early breast*  
539 *cancer*. Lancet, 1997. **349**(9055): p. 849.
- 540 34. Buchholz, T.A., et al., *Chemotherapy-induced apoptosis and Bcl-2 levels*  
541 *correlate with breast cancer response to chemotherapy*. Cancer J, 2003. **9**(1): p.  
542 33-41.
- 543 35. Arends, M.J. and A.H. Wyllie, *Apoptosis: mechanisms and roles in pathology*. Int  
544 Rev Exp Pathol, 1991. **32**: p. 223-54.
- 545 36. Mesner, P.W., Jr., Budihardjo, II, and S.H. Kaufmann, *Chemotherapy-induced*  
546 *apoptosis*. Adv Pharmacol, 1997. **41**: p. 461-99.
- 547 37. Kolios, M.C., et al., *Ultrasonic spectral parameter characterization of apoptosis*.  
548 Ultrasound Med Biol, 2002. **28**(5): p. 589-97.
- 549 38. Jain, R.K., *Normalization of tumor vasculature: an emerging concept in*  
550 *antiangiogenic therapy*. Science, 2005. **307**(5706): p. 58-62.
- 551 39. Dvorak, H.F., et al., *Identification and characterization of the blood vessels of*  
552 *solid tumors that are leaky to circulating macromolecules*. Am J Pathol, 1988.  
553 **133**(1): p. 95-109.
- 554 40. Gilbert, L.A. and M.T. Hemann, *DNA damage-mediated induction of a*  
555 *chemoresistant niche*. Cell, 2010. **143**(3): p. 355-66.
- 556 41. Junttila, M.R. and F.J. de Sauvage, *Influence of tumour micro-environment*  
557 *heterogeneity on therapeutic response*. Nature, 2013. **501**(7467): p. 346-54.
- 558 42. Hashizume, H., et al., *Openings between defective endothelial cells explain*  
559 *tumor vessel leakiness*. Am J Pathol, 2000. **156**(4): p. 1363-80.
- 560 43. Damia, G. and S. Garattini, *The pharmacological point of view of resistance to*  
561 *therapy in tumors*. Cancer Treat Rev, 2014. **40**(8): p. 909-16.
- 562 44. Minchinton, A.I. and I.F. Tannock, *Drug penetration in solid tumours*. Nat Rev  
563 Cancer, 2006. **6**(8): p. 583-92.
- 564 45. Jain, R.K., *Normalizing tumor microenvironment to treat cancer: bench to*  
565 *bedside to biomarkers*. J Clin Oncol, 2013. **31**(17): p. 2205-18.
- 566 46. Eisenhauer, E.A., et al., *New response evaluation criteria in solid tumours:*  
567 *revised RECIST guideline (version 1.1)*. Eur J Cancer, 2009. **45**(2): p. 228-47.
- 568 47. O'Flynn, E.A. and N.M. DeSouza, *Functional magnetic resonance: biomarkers of*  
569 *response in breast cancer*. Breast Cancer Res, 2011. **13**(1): p. 204.
- 570 48. Belli, P., et al., *Diffusion-weighted imaging in evaluating the response to*  
571 *neoadjuvant breast cancer treatment*. Breast J, 2011. **17**(6): p. 610-9.
- 572 49. Craciunescu, O.I., et al., *DCE-MRI parameters have potential to predict response*  
573 *of locally advanced breast cancer patients to neoadjuvant chemotherapy and*  
574 *hyperthermia: a pilot study*. Int J Hyperthermia, 2009. **25**(6): p. 405-15.
- 575 50. Jiang, L., et al., *Blood oxygenation level-dependent (BOLD) contrast magnetic*  
576 *resonance imaging (MRI) for prediction of breast cancer chemotherapy*  
577 *response: a pilot study*. J Magn Reson Imaging, 2013. **37**(5): p. 1083-92.

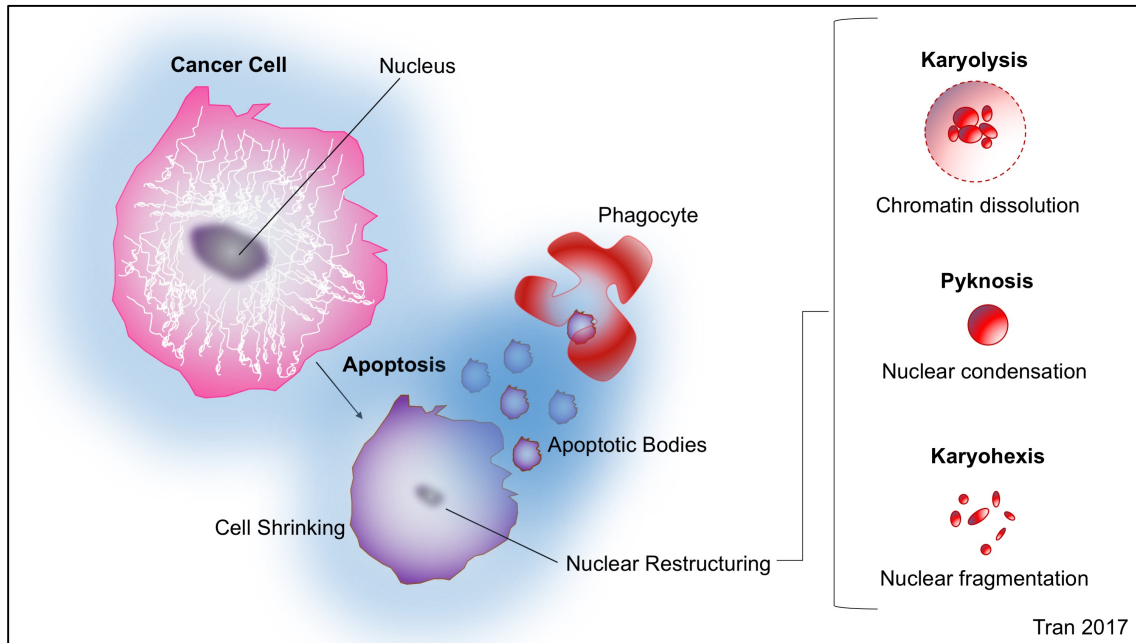
- 578 51. Andrade, W.P., et al., *Can FDG-PET/CT predict early response to neoadjuvant*  
579 *chemotherapy in breast cancer?* Eur J Surg Oncol, 2013. **39**(12): p. 1358-63.
- 580 52. Lodge, M.A., et al., *Parametric images of blood flow in oncology PET studies*  
581 *using [<sup>15</sup>O]water.* J Nucl Med, 2000. **41**(11): p. 1784-92.
- 582 53. Duch, J., et al., *<sup>18</sup>F-FDG PET/CT for early prediction of response to neoadjuvant*  
583 *chemotherapy in breast cancer.* Eur J Nucl Med Mol Imaging, 2009. **36**(10): p.  
584 1551-7.
- 585 54. Roblyer, D., et al., *Optical imaging of breast cancer oxyhemoglobin flare*  
586 *correlates with neoadjuvant chemotherapy response one day after starting*  
587 *treatment.* Proc Natl Acad Sci U S A, 2011. **108**(35): p. 14626-31.
- 588 55. Cerussi, A., et al., *Predicting response to breast cancer neoadjuvant*  
589 *chemotherapy using diffuse optical spectroscopy.* Proc Natl Acad Sci U S A,  
590 2007. **104**(10): p. 4014-9.
- 591 56. Jiang, S., et al., *Predicting breast tumor response to neoadjuvant chemotherapy*  
592 *with diffuse optical spectroscopic tomography prior to treatment.* Clin Cancer  
593 Res, 2014. **20**(23): p. 6006-15.
- 594 57. Cerussi, A., et al., *In vivo absorption, scattering, and physiologic properties of 58*  
595 *malignant breast tumors determined by broadband diffuse optical spectroscopy.*  
596 J Biomed Opt, 2006. **11**(4): p. 044005.
- 597 58. Cerussi, A., et al., *Diffuse optical spectroscopic imaging correlates with final*  
598 *pathological response in breast cancer neoadjuvant chemotherapy.* Philos Trans  
599 A Math Phys Eng Sci, 2011. **369**(1955): p. 4512-30.
- 600 59. Mourant, J.R., et al., *Light scattering from cells: the contribution of the nucleus*  
601 *and the effects of proliferative status.* J Biomed Opt, 2000. **5**(2): p. 131-7.
- 602 60. Fantini, S. and A. Sassaroli, *Near-infrared optical mammography for breast*  
603 *cancer detection with intrinsic contrast.* Ann Biomed Eng, 2012. **40**(2): p. 398-  
604 407.
- 605 61. Ueda, S., et al., *Baseline tumor oxygen saturation correlates with a pathologic*  
606 *complete response in breast cancer patients undergoing neoadjuvant*  
607 *chemotherapy.* Cancer Res, 2012. **72**(17): p. 4318-28.
- 608 62. Schrader, J., et al., *Matrix stiffness modulates proliferation, chemotherapeutic*  
609 *response, and dormancy in hepatocellular carcinoma cells.* Hepatology, 2011.  
610 **53**(4): p. 1192-205.
- 611 63. Wells, P.N. and H.D. Liang, *Medical ultrasound: imaging of soft tissue strain and*  
612 *elasticity.* J R Soc Interface, 2011. **8**(64): p. 1521-49.
- 613 64. Hayashi, M., et al., *Evaluation of tumor stiffness by elastography is predictive for*  
614 *pathologic complete response to neoadjuvant chemotherapy in patients with*  
615 *breast cancer.* Ann Surg Oncol, 2012. **19**(9): p. 3042-9.
- 616 65. Evans, A., et al., *Can shear-wave elastography predict response to neoadjuvant*  
617 *chemotherapy in women with invasive breast cancer?* Br J Cancer, 2013.  
618 **109**(11): p. 2798-802.
- 619 66. Martinoli, C., et al., *Power Doppler sonography: general principles, clinical*  
620 *applications, and future prospects.* Eur Radiol, 1998. **8**(7): p. 1224-35.
- 621 67. Czarnota, G.J., et al., *Ultrasound imaging of apoptosis: high-resolution non-*  
622 *invasive monitoring of programmed cell death in vitro, in situ and in vivo.* Br J  
623 Cancer, 1999. **81**(3): p. 520-7.



- 624 68. Sadeghi-Naini, A., et al., *Early prediction of therapy responses and outcomes in*  
625 *breast cancer patients using quantitative ultrasound spectral texture*. *Oncotarget*,  
626 2014. **5**(11): p. 3497-511.
- 627 69. Tadayyon, H., et al., *A priori Prediction of Neoadjuvant Chemotherapy Response*  
628 *and Survival in Breast Cancer Patients using Quantitative Ultrasound*. *Scientific*  
629 *Reports*, 2017. **7**: p. 45733.
- 630 70. Martincich, L., et al., *Variation of breast vascular maps on dynamic contrast-*  
631 *enhanced MRI after primary chemotherapy of locally advanced breast cancer*.  
632 *AJR Am J Roentgenol*, 2011. **196**(5): p. 1214-8.
- 633 71. Fan, B., et al., *Blood oxygen level-dependent MRI for the monitoring of*  
634 *neoadjuvant chemotherapy in breast carcinoma: initial experience*. *Magn Reson*  
635 *Imaging*, 2011. **29**(2): p. 153-9.
- 636 72. Padhani, A.R., *Functional MRI for anticancer therapy assessment*. *Eur J Cancer*,  
637 2002. **38**(16): p. 2116-27.
- 638 73. Baek, H.M., et al., *Predicting pathologic response to neoadjuvant chemotherapy*  
639 *in breast cancer by using MR imaging and quantitative 1H MR spectroscopy*.  
640 *Radiology*, 2009. **251**(3): p. 653-62.
- 641 74. Tozaki, M., et al., *Predicting pathological response to neoadjuvant chemotherapy*  
642 *in breast cancer with quantitative 1H MR spectroscopy using the external*  
643 *standard method*. *J Magn Reson Imaging*, 2010. **31**(4): p. 895-902.
- 644 75. Mankoff, D.A., et al., *Blood flow and metabolism in locally advanced breast*  
645 *cancer: relationship to response to therapy*. *J Nucl Med*, 2002. **43**(4): p. 500-9.
- 646 76. Cerussi, A., et al., *Sources of absorption and scattering contrast for near-infrared*  
647 *optical mammography*. *Acad Radiol*, 2001. **8**(3): p. 211-8.
- 648 77. Intes, X., *Time-domain optical mammography SoftScan: initial results*. *Acad*  
649 *Radiol*, 2005. **12**(8): p. 934-47.
- 650 78. Shia, W.C., et al., *Effectiveness of evaluating tumor vascularization using 3D*  
651 *power Doppler ultrasound with high-definition flow technology in the prediction of*  
652 *the response to neoadjuvant chemotherapy for T2 breast cancer: a preliminary*  
653 *report*. *Phys Med Biol*, 2015. **60**(19): p. 7763-78.
- 654 79. Lizzi, F.L., et al., *Statistical framework for ultrasonic spectral parameter imaging*.  
655 *Ultrasound Med Biol*, 1997. **23**(9): p. 1371-82.
- 656 80. Hunt, J.W., et al., *A model based upon pseudo regular spacing of cells combined*  
657 *with the randomisation of the nuclei can explain the significant changes in high-*  
658 *frequency ultrasound signals during apoptosis*. *Ultrasound Med Biol*, 2002. **28**(2):  
659 p. 217-26.
- 660 81. Haralick, R.M., K. Shanmugam, and I. Dinstein, *Textural features for image*  
661 *classification*. *IEEE Transactions on Systems, Man and Cybernetics*, 1973. **3**: p.  
662 610-621.
- 663 82. Davnall, F., et al., *Assessment of tumor heterogeneity: an emerging imaging tool*  
664 *for clinical practice?* *Insights Imaging*, 2012. **3**(6): p. 573-89.
- 665 83. Li, H., et al., *Computerized texture analysis of mammographic parenchymal*  
666 *patterns of digitized mammograms*. *Acad Radiol*, 2005. **12**(7): p. 863-73.
- 667 84. Chen, W., et al., *Volumetric texture analysis of breast lesions on contrast-*  
668 *enhanced magnetic resonance images*. *Magn Reson Med*, 2007. **58**(3): p. 562-  
669 71.

- 670 85. Lerski, R.A., et al., *MR image texture analysis--an approach to tissue*  
671 *characterization*. Magn Reson Imaging, 1993. **11**(6): p. 873-87.
- 672 86. Chicklore, S., et al., *Quantifying tumour heterogeneity in 18F-FDG PET/CT*  
673 *imaging by texture analysis*. Eur J Nucl Med Mol Imaging, 2013. **40**(1): p. 133-40.
- 674 87. Yang, X., et al., *Ultrasound GLCM texture analysis of radiation-induced parotid-*  
675 *gland injury in head-and-neck cancer radiotherapy: an in vivo study of late*  
676 *toxicity*. Med Phys, 2012. **39**(9): p. 5732-9.
- 677 88. Castellano, G., et al., *Texture analysis of medical images*. Clin Radiol, 2004.  
678 **59**(12): p. 1061-9.
- 679 89. Gibbs, P. and L.W. Turnbull, *Textural analysis of contrast-enhanced MR images*  
680 *of the breast*. Magn Reson Med, 2003. **50**(1): p. 92-8.
- 681 90. Gupta, R. and P.E. Undrill, *The use of texture analysis to delineate suspicious*  
682 *masses in mammography*. Phys Med Biol, 1995. **40**(5): p. 835-55.
- 683 91. Sadeghi-Naini, A., et al., *Early detection of chemotherapy-refractory patients by*  
684 *monitoring textural alterations in diffuse optical spectroscopic images*. Med Phys,  
685 2015. **42**(11): p. 6130-46.
- 686 92. Ahmed, A., et al., *Texture analysis in assessment and prediction of*  
687 *chemotherapy response in breast cancer*. J Magn Reson Imaging, 2013. **38**(1):  
688 p. 89-101.
- 689 93. Gangeh, M.J., et al., *Computer Aided Theragnosis Using Quantitative Ultrasound*  
690 *Spectroscopy and Maximum Mean Discrepancy in Locally Advanced Breast*  
691 *Cancer*. IEEE Trans Med Imaging, 2016. **35**(3): p. 778-90.
- 692 94. Eccles, S.A., et al., *Critical research gaps and translational priorities for the*  
693 *successful prevention and treatment of breast cancer*. Breast Cancer Res, 2013.  
694 **15**(5): p. R92.
- 695 95. Cooper, R.S. and B.M. Psaty, *Genomics and medicine: distraction, incremental*  
696 *progress, or the dawn of a new age?* Ann Intern Med, 2003. **138**(7): p. 576-80.
- 697 96. Chin, L., J.N. Andersen, and P.A. Futreal, *Cancer genomics: from discovery*  
698 *science to personalized medicine*. Nat Med, 2011. **17**(3): p. 297-303.
- 699 97. Wang, L., H.L. McLeod, and R.M. Weinshilboum, *Genomics and drug response*.  
700 N Engl J Med, 2011. **364**(12): p. 1144-53.
- 701 98. Straver, M.E., et al., *The 70-gene signature as a response predictor for*  
702 *neoadjuvant chemotherapy in breast cancer*. Breast Cancer Res Treat, 2010.  
703 **119**(3): p. 551-8.
- 704 99. Paik, S., et al., *A multigene assay to predict recurrence of tamoxifen-treated,*  
705 *node-negative breast cancer*. N Engl J Med, 2004. **351**(27): p. 2817-26.
- 706 100. Paik, S., et al., *Gene expression and benefit of chemotherapy in women with*  
707 *node-negative, estrogen receptor-positive breast cancer*. J Clin Oncol, 2006.  
708 **24**(23): p. 3726-34.
- 709 101. Sparano, J.A., et al., *Prospective Validation of a 21-Gene Expression Assay in*  
710 *Breast Cancer*. N Engl J Med, 2015. **373**(21): p. 2005-14.
- 711 102. Carlson, J.J. and J.A. Roth, *The impact of the Oncotype Dx breast cancer assay*  
712 *in clinical practice: a systematic review and meta-analysis*. Breast Cancer Res  
713 Treat, 2013. **141**(1): p. 13-22.
- 714 103. O'Connor, J.P., et al., *Imaging biomarker roadmap for cancer studies*. Nat Rev  
715 Clin Oncol, 2016.

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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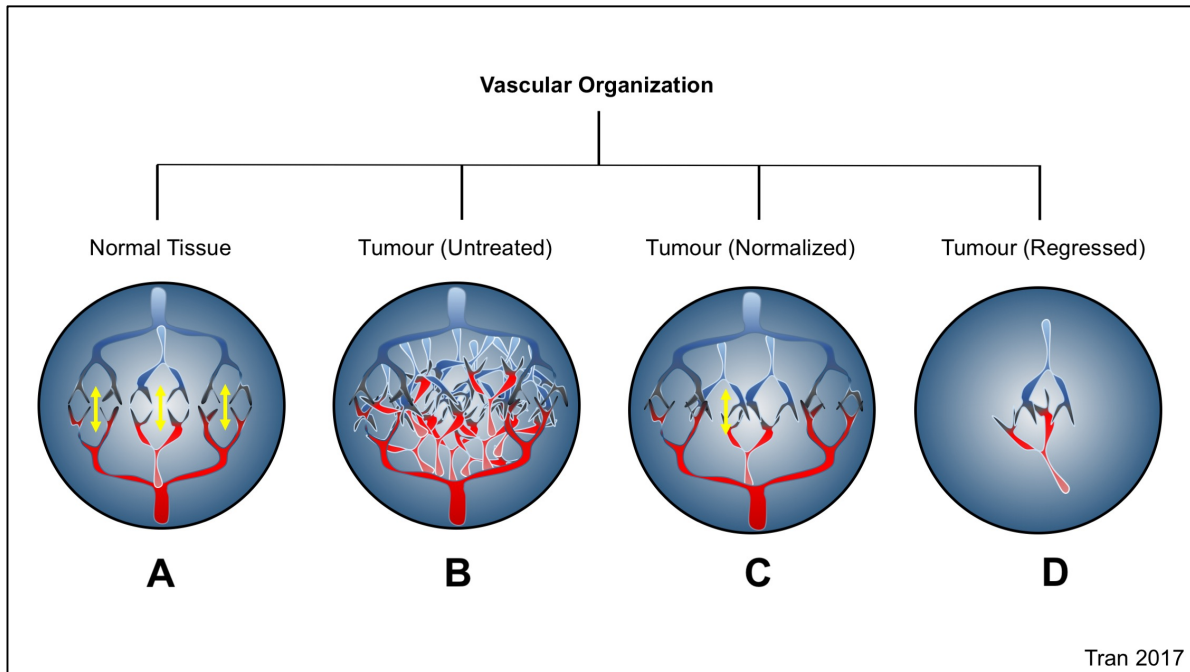
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735 **Figure 2**

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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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