Imaging Biomarkers for Precision Medicine in Locally Advanced Breast Cancer

TRAN, W T, CHILDS, Charmaine <http://orcid.org/0000-0002-1558-5633>, PROBST, Heidi <http://orcid.org/0000-0003-0035-1946>, FARHAT, G and CZARNOTA, G J

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.

Commentary: Imaging Biomarkers for Precision Medicine in Locally Advanced Breast Cancer

W. T. Tran[1,2,3,*], C. Childs[3], H. Probst[3], G. Farhat[1,2], G. J. Czarnota[1,2]

[1]Department of Radiation Oncology, Sunnybrook Health Sciences Centre, Toronto, Canada
[2]Physical Sciences, Sunnybrook Research Institute, Toronto, Canada
[3]Facutly of Health and Wellbeing, Sheffield Hallam University, Sheffield, United Kingdom

ACCEPTED FOR PUBLICATION TO JOURNAL OF MEDICAL IMAGING AND RADIATION SCIENCES: SEPTEMBER 18 2017

Keywords: Imaging Biomarkers, Chemotherapy response, Locally advanced breast cancer

Corresponding Author

William T. Tran
Department of Radiation Oncology
Sunnybrook Health Sciences Centre
Toronto, Ontario, Canada, M5S3K5
Tel: 416 480 6100 x 1099
Email: william.tran@sunnybrook.ca

ABSTRACT

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

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

INTRODUCTION AND BACKGROUND

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

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

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

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

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

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

HALLMARKS OF CHEMORESISTANCE AND CHEMOEFFICACY

Intertumor and Intratumor Heterogeneity Contributes to Chemoresistance

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

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

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

Mechanisms of Chemoefficacy

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

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

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

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

IMAGING BIOMARKERS AS INDICATORS FOR CHEMORESPONSE

Conventional Imaging Methods

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

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

Magnetic Resonance Imaging Biomarkers

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

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

Positron-Emission Tomography (PET)

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

demonstrates radioactive decay, permitting PET imaging to map metabolic activity in tissue. Increased FDG-uptake (standard uptake value, SUV) has been demonstrated in tumors since tumor metabolism is greater compared to normal tissue. PET imaging can, therefore, serve to identify the extent of malignancies [51]. PET imaging is achieved with the release of a gamma-ray photon that is detected by a photon-detection device during radioactive decay, known as positron-electron annihilation. Another radiotracer used in PET is the radionuclide ^{15}O-H_2O, which is used to measure tumor blood flow; where the distribution of water can be equated to blood activity in blood vessels [52]. Previous work from Duch et al. (2009) showed that the intratreatment change in SUV (ΔSUV, 2 cycles of chemotherapy) differentiated between pathologic response groups (responders vs. non-responders) with a sensitivity of 77% and specificity of 80%, using a cut-off value of 40% [53].

*Diffuse Optical Spectroscopy (DOS***)**

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

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

Ultrasound Imaging Biomarkers

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

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

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

Technique	Biomarker Measurements	Treatment Points Studied	Ref.
Magnetic Resonance Imaging			
DWI-MRI	• Extracellular water motion • Tumor-cell density • Tissue micro-structure • Cell membrane integrity • Cell membrane permeability	• Pre-treatment • Intratreatment • Post-chemotherapy	[48] [47] [47] [70] [71] [50] [72] [73] [74]
DCE-MRI	• Vascular permeability • Dynamic blood flow		
BOLD	• Tumor oxygenation • Tumor vascularity • Angiogenesis • Blood Volume • Blood Flow		
SPECT	• Reduction in mitotic count • Tumor cellularity • Cell membrane integrity • Tumor metabolism • Tissue composition (lipid)		
Positron-Emission Tomography			
^{18}F-FDG	• Tumor metabolism	• Pre-treatment • Intratreatment • Post-chemotherapy	[75] [52] [75]
^{15}O-H_2O	• Tumor blood flow		
Diffuse Optical Spectroscopy			
DOS	• Metabolism • Cell activity • Vascular Density • Edema • Breast tissue composition • Cellularity • Cell death and Morphology • Tissue contrast • Hypoxia	• Pre-treatment • Intratreatment • Post-chemotherapy	[57] [76] [58] [77] [60] [57] [60] [60]
Ultrasound			
Elastography	• Tumor progression • Extracellular matrix • Collagen crosslinking • Tissue composition (fibrosis)	• Pre-treatment • Intratreatment • Post-chemotherapy	[64] [62] [65] [78] [79] [79] [80]
Power Doppler	• Vascular blood flow • Blood perfusion • Vascularity		
QUS	• Tumor Cell Death (Apoptosis) • Cell Morphology and Distribution		

Table 1. Imaging biomarker studies have included MRI, PET imaging, DOS, and ultrasound based imaging. The studies have included response assessment using various biological features at various stages of chemotherapy treatment: before 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

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

STATUS OF IMAGING BIOMARKERS FOR PERSONALIZED MEDICINE IN BREAST CANCER

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

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

considerations include optimizing the treatment time-course from individual tumor and patient data. Currently, overtreatment is a clinical challenge.

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

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

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

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

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

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

REFERENCES

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

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

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

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

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

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

Figure 1

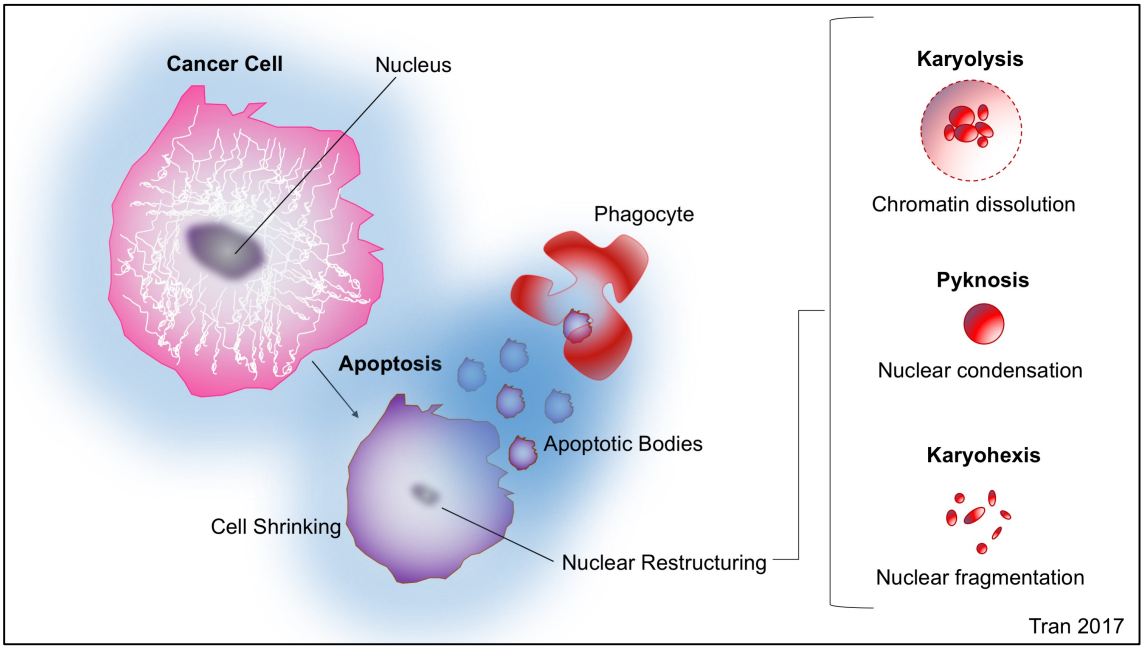

Figure 1: Apoptosis in cancer cells. Apoptosis is characterized as an energy dependent mechanism where cells undergo programmed morphological changes. Chemotherapies induce apoptosis in tumor cells and this results in cell shrinking and nuclear restructuring such as karyolysis, pyknosis and karyorhexis.

Figure 2

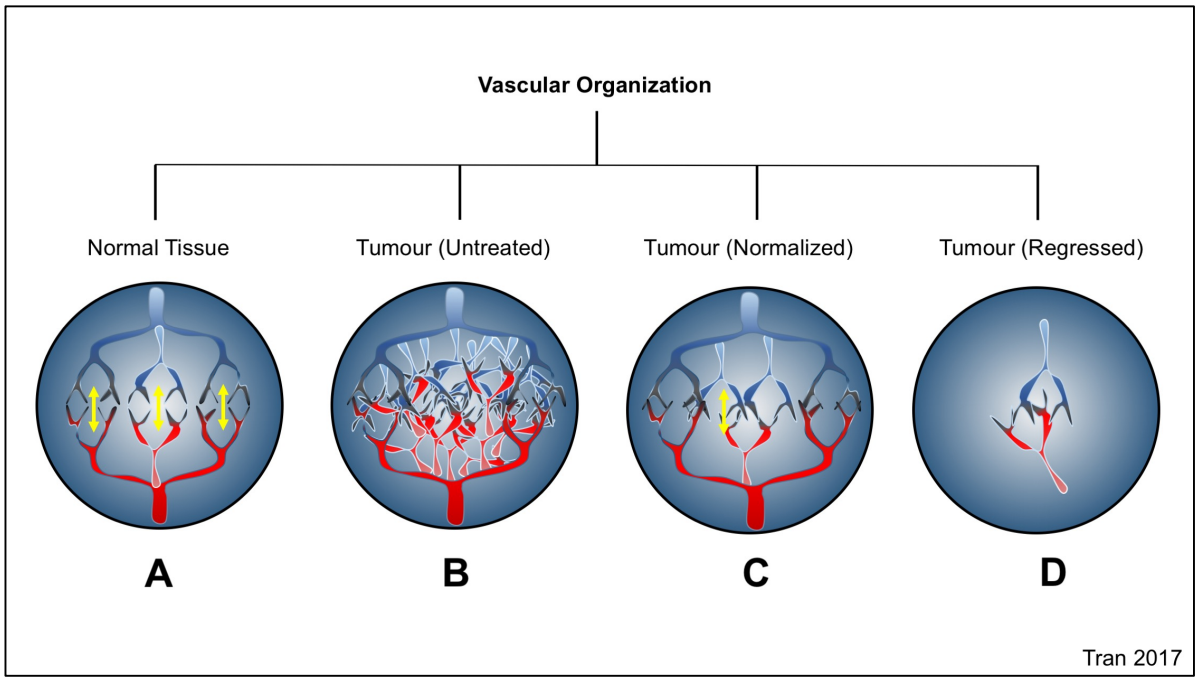

Figure 2: A comparison of the vascular organization. **A.** Normal tissue exhibits well-organized vasculature, which permit exchange of biomolecules and gas (arrows). **B.** Untreated tumors show high density vasculature and do not permit free exchange of biomolecules and gasses. **C.** Normalized tumors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