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Targeted drugs and diagnostic assays: Companions in the race to combat ethnic disparity

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1. ABSTRACT

African Americans (AAs) are more likely than European Americans to develop aggressive breast cancer subtypes, and have higher recurrence and mortality rates; this results in a stark breast-cancer related ethnic disparity in clinical outcomes. In this era of personalized oncology, companion diagnostics (CDx) are transforming the cancer treatment narrative slowly but steadily, by enabling the use of safety and/or efficacy biomarkers to stratify patient populations, and thus ensuring more effective deployment of targeted therapeutics. This parallel co-development of drugs and *in vitro* diagnostic assays is turning out to be the cornerstone of individualized cancer treatment. In this review, we assert that development of drugs and CDx targeted towards molecular and centrosomal aberrations that occur more frequently in AAs could yield next generation precision medicine tools better informed and inspired by, and more finely attuned to the unique tumor biology of AAs. By understanding more deeply ancestry-associated differences among breast tumors of different ethnicities, and gearing our drug and CDx development efforts to target these distinctions, we might be able to significantly alleviate racial health disparity.

2. INTRODUCTION

Recent advancements in our understanding of molecular alterations underlying cancer have led to the practice of oncology going through its biggest shakeup ever, as it morphs and adopts a more precise and personalized model of cancer treatment. This change in narrative has been spurred by the combined winds of pharmaceutical industry and scientific innovation focusing on developing therapies that target specific genetic changes in cancer cells. The rapid development of several novel cancer biomarkers and targeted drugs has thus birthed a fresh approach to cancer drug discovery and development. Appropriately termed, companion diagnostics (CDx), the novel tactic considers the uniqueness of each patient's disease by using *in vitro* clinical laboratory assays to detect and target tumor gene alterations to customize drug selection and administration. This innovative strategy of personalizing patient care enhances efficacy of conventional cancer treatment practices as each patient has often has unique gene mutations that contribute to disease onset and/or arise concomitant with tumor progression and therapeutic interventions (1). Stratification of patients based on their unique biomarker complement and customization

of treatment is postulated to make economic sense too as it trims down healthcare costs and improves outcomes for patients (2). The monoclonal antibody, trastuzumab, has already been U.S. Food and Drug administration (FDA)-approved as a targeted therapy for patients with breast tumors overexpressing HER2, and KRAS mutation testing is currently being conducted in clinics before prescribing cetuximab for patients with colorectal cancers (3). Vemurafenib has had clinical success in targeting the BRAF V600 mutation in metastatic melanomas and crizotinib has attenuated tumor activity in non-small cell lung cancers through targeting the EML 4-ALK mutation (4). Statistics show that approximately two-thirds of the recent breakthrough therapy designations granted by the FDA include an accompanying diagnostic assay (5). Thus, the clinical application of genomic technologies and companion *in vitro* diagnostic assays is quickly revolutionizing the clinical oncology landscape into a gene-based bed of better-optimized, more efficacious and potentially cost-saving therapies for cancer patients.

Despite impressive improvements in cancer care in the past few decades, substantial disparities in outcomes persist between breast cancer patients of African and European ancestry in the clinic. Typically, African-American (AA) breast tumors harbor inherently more aggressive phenotypes and therefore progress more rapidly to metastatic disease; this unfortunately precipitates considerably higher recurrence and mortality rates in AA patients compared to their European-American (EA) counterparts (6, 7). This feature of AA tumors has spawned several studies investigating the genetic profile of AA breast tumors, to obtain deeper insights into the notable distinctions in tumor biology between AA and EA breast tumors. Accumulating gene expression microarray and immunohistochemical evidence have revealed a considerable number of differentially expressed genes among AA and EA breast tumors that have been observed to correlate with tumor aggressiveness markers such as high Ki67 Index of proliferation, lymph node metastasis, larger tumor size, and poor differentiation status. Accompanying these differences in gene expression are numerous alterations in gene copy number, gene polymorphisms, and epigenetic modifications among ethnically distinct breast tumors that are believed to also concertedly contribute to the racial inequalities in disease progression (8-10). Thus, targeting these molecular distinctions as therapeutics for breast cancer patients of African descent in the clinic may be fundamental in counteracting their aggressive disease course. Herein, we discuss how targeted drugs and their CDx may offer (a) significant promise for bridging the racial disparity in breast cancer outcomes by proffering AA breast cancer patients with treatments tailored toward their distinctive tumor biological characteristics and (b) address concerns about cost-effectiveness of personalized medicine.

3. TAILORING THERAPY: COMPANION DIAGNOSTICS ENABLE MORE PRECISE TARGETING OF THERAPEUTICS FOR BREAST CANCER PATIENTS

As eloquently stated by the FDA's deputy commissioner for medical products and tobacco, Stephen Spielberg, M.D., Ph.D., today's challenge in devising innovative therapeutics stems from "recognizing the huge human diversity in the causes of disease and in the response to medicines and other interventions. It's figuring out the true biological basis of the diseases, increasing diagnostic precision and developing and using medicines targeted at specific causes of disease." This quote impeccably captures the essence of the revolutionary movement of medicine towards a more personalized approach owing to strong heterogeneity in clinical presentation and inherent tumor biology of cancers that make efficacious treatment of the disease a prohibitive challenge.

Chemotherapy has long been the "go-to" option for breast cancer treatment, along with radiation and hormonal therapies (11). However, owing to (i) adverse side-effects of these cytotoxic chemotherapeutic agents that do not sharply distinguish between malignant and healthy cells, and (ii) recent discoveries of specific molecular aberrations underlying the heterogeneity of breast tumors, targeted treatments have surfaced as a potentially more selective, and thus, superior alternative (Table 1) that may offer an edge in the battle against this formidable disease (12). This philosophy has provided an impetus for breast cancer researchers to identify novel biomarkers whose assessment may allow them to determine the distinct biomarker "fingerprint" of individual tumors and identify the most optimal therapy, paving the path for the catalyst of "stratified" medicine. From these novel discoveries, a two-pronged approach has arisen for the proper implementation of personalized medicine in the clinic in which a drug is developed against a specific target and an assay is devised to accurately detect and/or measure a marker that predicts response to this drug. In layman's terms, the drug and the assay become "companions" in medicine to pose a more robust front in the course of treatment, underlying the foundation of Companion Diagnostics (CDx).

The universally accepted paradigm for CDx development is the drug-diagnostic co-development cooperative model in which the diagnostic exam is developed in parallel with the drug (13). This prototype was propagated from the 1990's success of trastuzumab, widely known as Herceptin, and its corresponding diagnostic immunohistochemistry (IHC) assay, HercepTest, designed by Dako (14). CDx assays are currently the cornerstones of personalized medicine and function as "gatekeepers" that enable stratification of patients to discern patient benefit from the drug in question, thus guiding clinical

Table 1. FDA approved CDx therapeutics for breast cancer

Drug	Target	Mechanism	CDx
Affinitor (Everolimus)	mTOR kinase in advanced hormone receptor positive and HER2 negative breast cancer in postmenopausal women; used in combination with exemestane	mTOR (mammalian target of rapamycin) kinase inhibitor that decreases rate of tumor growth and spread by blocking their energy availability	ER and PR IHC assay; HercepTEST (Dako), HER2 FISH pharmDx (Dako)
Tamoxifen (Nolvadex)	ER in ER+receptor positive breast cancer	Interferes with estrogen hormone binding to its receptor to impede growth of breast cancer cells	ER IHC assay
Toremifene (Fareston)	ER in ER+receptor positive breast cancer	Antagonizes ER to impede growth of breast cancer cells	ER IHC assay
Trastuzumab (Herceptin)	HER2 receptor for HER2+breast cancer	Blocks HER2 receptors on surface of breast cancer cells to prevent them from receiving growth signals	HercepTEST (Dako), HER2 FISH pharmDx (Dako)
Fulvestrunt (Fasoldex)	ER receptor for ER+metastatic breast cancer	Antagonizes ER to impede growth of breast cancer cells	ER IHC assay
Anastrozole (Arimidex)	Aromatase in postmenopausal ER+breast cancer	Impedes production of estrogen by interfering with the enzyme aromatase which converts androgen into estrogen	ER IHC assay
Exemestane (Aromasin)	Aromatase in postmenopausal ER+breast cancer	Impedes production of estrogen by interfering with the enzyme aromatase which converts androgen into estrogen	ER IHC assay
Lapatinib (Tykerb)	HER2 kinases in metastatic HER2+breast cancer; used in combination with xeloda or letrozole in postmenopausal women	Interferes with HER2-related kinases to limit access of breast cancer cells to energy needed for them to grow and thrive	HercepTEST (Dako), HER2 FISH pharmDx (Dako)
Letrozole (Femara)	Aromatase in postmenopausal HER2+breast cancer	Impedes production of estrogen by interfering with the enzyme aromatase which converts androgen into estrogen	HercepTEST (Dako), HER2 FISH pharmDx (Dako)
Pertuzumab (Perjeta)	HER2 receptor in metastatic HER2+breast cancer; used in combination with trastuzumab and docetaxel	Blocks HER2 receptors on surface of breast cancer cells to prevent them from receiving growth signals	HercepTEST (Dako), HER2 FISH pharmDx (Dako)
Ado-trastuzumab emtansine (Kadcyla)	HER2 receptor in metastatic HER2+breast cancer; used in patients previously treated with trastuzumab and taxane	Attaches to trastuzumab to bring emtansine to HER-2+cancer cells	HercepTEST (Dako), HER2 FISH pharmDx (Dako)
Palbociclib (Ibrance)	CDK4/6 in ER+and HER2- breast cancer; used in combination with letrozole	Cyclin-dependent kinase 4/6 inhibitor that interferes with cell division to impede cancer cell division	ER IHC assay; HercepTEST (Dako), HER2 FISH pharmDx (Dako)
Bevacizumab (Avastin)	Blood vessels in metastatic HER2- breast cancer	Impede growth of blood vessels that facilitate cancer growth	HercepTEST (Dako), HER2 FISH pharmDx (Dako)

treatment decisions. Like all clinical assays, CDx assays must be highly robust, reproducible, specific and sensitive prompting these diagnostic tests and their companion drugs to require sanction by the FDA, pre-market approvals, and laboratory-developed tests (LDTs) conducted in CLIA-accredited laboratories prior to assimilation into routine clinical practice (15). In most cases, one or more gene mutations that determine a patient's response to a given therapeutic agent are assessed and used to decide whether the patient should receive the agent in question. For example, *EML4-ALK* translocation is determined preceding the administration of ALK inhibitors in non-small

cell lung cancers and BRAF sequencing is conducted to predict clinical response to BRAF inhibitors in metastatic melanomas (1). Hence, CDx assays serve as a "decisive" stratification factor in the clinic to foretell if a patient will benefit from the recommended targeted therapeutic as illustrated in the following examples of successful deployment of CDx in the oncology clinic.

3.1. HercepTest and HER2 FISH

Herceptin/Trastuzumab has emerged as one of the most widely-administered first-line targeted therapies for breast cancer in clinics today. A recombinant monoclonal antibody, Trastuzumab, targets the product of the HER2 gene, which encodes for the growth factor receptor HER2, and is amplified in 25 to 30% of breast cancer cases to stimulate tumor progression and aggressiveness (16). Both HercepTest and HER2 FISH pharmDx kits, manufactured by Dako, have been FDA-approved as CDx assays to select patients for Herceptin treatment (17). By halting or drastically stunting tumor growth and decreasing the death rate from HER2-positive metastatic breast cancers by up to 20%, Herceptin improves efficacy of first-line chemotherapeutics when administered as neoadjuvant or adjuvant therapy (16). Furthermore, Herceptin administered concurrently with standard chemotherapy has been shown to improve rate and duration of response and improve overall survival (OS) (16). Consequently, all breast cancer patients are tested for HER2/neu status upon initial diagnosis by immunohistochemical staining of biopsies (16).

Pertuzumab (Perjeta), another monoclonal antibody, has also been FDA-approved as first-line treatment of HER2-positive breast cancers. A randomized controlled study has proven pertuzumab to reduce the rate of HER2-positive metastatic tumors when combined with trastuzumab and conventional chemotherapy and has been shown to boost survival more than trastuzumab and chemotherapy alone (18, 19). Another class of drugs implicated in the first-line treatment of HER2/neu positive metastatic breast cancers are tyrosine-kinase inhibitors, such as the FDA-approved lapatinib (Tykerb). Lapatinib, targets and interferes with tyrosine-kinase enzyme activity instrumental in tumor growth. In collaboration with chemotherapeutic drugs, such as paclitaxel, lapatinib can decrease the rate of metastasis in HER2/neu positive breast cancers patients (20, 21). HercepTest and HER2 FISH pharmDx tests also serve as reputable companion assays for pertuzumab and lapatinib.

3.2. Immunohistochemical assay for estrogen receptor alpha

Perhaps the longest administered CDx for breast cancer that may have paved the path for the rise of stratified medicine is the estrogen receptor (ER) assay to identify patients who would benefit from tamoxifen, an ER antagonist (22). Developed in the 1970's, tamoxifen was designed to combat ER-positive breast cancers, which comprise 70% of breast tumors (22). The IHC-based assay for ER alpha to identify patients for first-line tamoxifen treatment yielded a high degree of correlation between patient response and positive test results in a phase II clinical trial in 1976, sparking the launch of the first successful CDx assay (23). Close on the heels of the resounding success of this ER-targeted therapeutic, additional targeted therapies (such as aromatase inhibitors) for ER-positive breast tumors have since been brought into the fold of clinical use (22).

Targeting angiogenesis promoting factors has been high on the list in breast cancer drug discovery and development as angiogenesis plays a fundamental role in facilitating tumor growth and invasion (24). Agents are already being designed and in clinical trials to target the key pro-angiogenic factor, vascular endothelial growth factor (VEGF), which induces vascular permeability and vessel formation (24). VEGF has been observed to be overexpressed in lymph node positive and negative breast cancers displaying poor clinical prognoses (24). Clinical studies have achieved significant success in inhibiting VEGF expression in metastatic breast cancer with the popular VEGF inhibitor, Bevacizumab (Avastin) (25). A humanized monocolonal antibody, bevacizumab, targets all isoforms of VEGF-A and has reaped success in the landmark E2100 randomized trial in which bevacizumab administered simultaneously with paclitaxel prompted a significant increase in overall response rate and progression free survival (PFS) than paclitaxel alone in metastatic breast cancer (26). Recent studies have suggested that VEGF single nucleotide polymorphisms may serve as markers for patient response to bevacizumab (27). However, there remains an urgent need for clinically validated robust biomarkers that can suitably guide patient selection for anti-VEGF therapy in the clinic (25). Studies that can identify surrogate markers for VEGF expression in tumor tissue, bodily fluids, circulating endothelial cells, or tumor interstitial fluid pressure through modern imaging techniques are urgently needed for optimal clinical performance of VEGF inhibitors (25).

As illustrated above in the stark shift away from the traditional "one size fits all" paradigm, discriminatory medicine that precisely "customizes a shoe for each patient" is slowly and surely revolutionizing breast cancer treatment. Indeed, studies that demonstrate significantly-improved clinical outcomes when treatments are targeted more precisely, have designated targeted therapies as an imperative strategy in clinical oncology.

4. NO PATIENT LEFT BEHIND: COULD PERSONALIZED MEDICINE PERHAPS ALLEVIATE THE RACIAL DISPARITY IN BREAST CANCER OUTCOMES?

Breast cancer patients of African ancestry continue to be left behind in the battle against breast

Table 2. Reported therapeutics in clinical trials targeting inherent tumor biological characteristics of AA breast tumors

Therapeutic (s)	Target	Differential expression in AA	Activity	Suggested CDx
Poly (ADP-ribose) polymerase-1 (PARP-1) inhibitors	BRCA1/2	Overexpressed	Selectively destroys cancer cells harboring BRCA1 or 2 mutations	BRCA gene mutation screening; PARP1 IHC assay; Biomarkers predicting sensitivity to PARP1 inhibition
p53 re-activation and induction of massive apoptosis (PRIMA-1); Adenovirus-based therapies	p53	Overexpressed	Convert mutant p53 into its functional form to stimulate apoptosis	p53 IHC assay; gene sequencing
MMP inhibitors	Matrix metalloproteinase (MMPs)	Overexpressed	Inhibit expression of MMPs that promote tumor metastasis	MMP IHC assay; biological imaging
EGFR inhibitors (i.e. cetuximab)	Epidermal growth factor (EGFR)	Overexpressed in TNBC	Stimulates anti-tumor activity when combined with chemotherapeutics	EGFR IHC assay potentially combined with KRAS mutation testing

cancer as overwhelming evidence reveals their mortality rates remain higher than the corresponding rates for other ethnicities (28). In the US, although, conventional therapeutic approaches have reaped moderate success for EA breast cancer patients, standard clinical treatment options appear to be falling short in the treatment of the aggressive tumor phenotypes harbored by many AA breast cancer patients. The disparate survival rates and treatment response among patients of different ethnicities has recently been explicated by mounting evidence supporting differences in gene expression profiles and inherent tumor biology linked to their ancestry. With the previously discussed increasing evidence advocating targeted therapies as enhancing efficacy of first-line chemotherapeutics and a cornucopia of inherent biological disparities underpinning racial disparities in clinical outcomes, personalized medicine is rapidly gaining ammunition as a compelling new game plan in combating the challenges presented by AA breast tumors.

Interestingly, many of the existing efforts in the development of targeted therapies for breast cancer are in fact, inadvertently targeting genetic targets of AA cancer cells (Table 2). Recent studies have discovered that mutations in the familial breast cancer genes, BRCA1 and BRCA2, which confer on women a predisposition to breast cancer, are a frequent occurrence in breast cancer patients of African ancestry (29). A high frequency of mutations in the BRCA2 gene has also been linked to earlier onset of breast cancer in AA women and BRCA1 mutations have been strongly associated with breast tumors of the highly aggressive triple-negative and basal-like phenotypes (30, 31). Therapeutic approaches targeting BRCA1/2 mutations are already a large area of focus in clinical oncologic research with developments in inhibiting the nuclear enzyme, poly (ADP-ribose) polymerase-1 (PARP-1) showing promise (32). BRCA1/2 proteins play a chief role in repair of double-stranded DNA breaks (33). PARP-1 also plays a key role in the base excision repair pathway by aiding in repair of single-strand DNA breaks (24). However, inhibition of PARP-1 can generate numerous double-stranded DNA breaks (34). Thus, PARP-1 inhibitors in preclinical studies have made remarkable strides in selectively killing cancer cells harboring BRCA1/2 mutations, which lack the ability to repair double-stranded breaks, consequently leading to cell death (24). The intravenously administered PARP-1 inhibitor, Iniparib (BSI-201), has demonstrated noteworthy antitumor activity when combined with chemotherapeutics in preclinical *in vitro* and in vivo studies by improving PFS and OS in metastatic triple negative breast cancer (TNBC) patients (24). Although no clinically validated methods of selecting patients most likely to exhibit a robust response to PARP-1 inhibitors exist, standard BRCA genetic mutation screening routinely conducted in clinics may suffice for predicting a significant response in AA breast cancer patients. Discoveries of biomarkers that can predict patient response may impart insights into sensitivity of AA breast cancer patients to PARP inhibitors (35).

Mutations in the p53 tumor suppressor gene have long been acknowledged to serve a central role in the onset of most cancers, promotion of tumor vitality, and conferment of therapeutic resistance (24). Recent studies have reported considerably higher p53 expression in AA breast tumors than EA breast tumors, potentially explaining differences in patient outcomes among the ethnicities (36, 37). Conveniently, studies are already underway to restore p53 function in breast tumors in order to induce apoptosis of tumor cells (24). *In vitro* and *in vivo* experiments with non-toxic small molecules called p53 re-activation and induction of massive apoptosis

(PRIMA-1) that convert mutant p53 (mtp53) into its functional form, triggered apoptosis of mtp53-expressing breast cancer cell lines, BT-474, HCC-1428, and T47-D (24). The tumor regressive abilities of PRIMA-1 launches PRIMA-1 as a potential anti-cancer therapeutic agent for AA breast cancer patients expressing mutant p53. Despite compelling evidence supporting p53 mutations as bearing a significant impact on survival outcomes and response to treatment, there lingers a strong lack of consensus on concrete detection methods for the biomarker that can be suitable in a clinical setting owing to inconsistencies in immunohistochemical test results (38). Gene sequencing offers a more reliable source of p53 mutation detection but presents the drawbacks of not being affordable and practicable for routine clinical use (38). Therefore, overcoming these significant limitations would be essential before therapeutics targeting p53 mutations can be assimilated into the clinic for the benefit of AA breast cancer patients.

Matrix metalloproteinases (MMPs) have recently been added to the lengthy list of mutated genes in AA breast cancer patients that are believed to facilitate the increased propensity of AA breast tumors to metastasize compared to EA breast tumors (39). Expression of MMP proteins has been observed to be overexpressed in AA breast cancer cell lines compared to Caucasian cell lines (39). As endoproteinases that degrade the extracellular matrix (ECM), MMPs bestow cancer cells with invasive capabilities and thus, have impelled cancer researchers to devise strategies for MMP inhibition (24). Development of MMP inhibitors has long been underway launched by the phase I study involving tanomastat (BAY 12-9566), an inhibitor of MMP-2, MMP-3, and MMP-9, which was successfully tolerated in patients harboring solid tumors (40). More clinical trials are currently being conducted to assess viability of additional newly designed MMP inhibitors (24). If efficacious in clinical trials, MMP inhibitors may theoretically impede robust metastatic tendencies frequently displayed by AA breast tumors. Clinically validated methods of assessing MMP expression (in tumor cells and/or stromal cells and in the ECM) as companions for MMP inhibitors will be imperative for successful routine clinical use of this novel targeted therapeutic for AA breast cancer patients.

Epidermal growth factor receptor (EGFR) gene is highly amplified in TNBCs (Anders and Carey, 30). Given the high prevalence of TNBC among AA breast cancer patients, therapeutics targeting this aberrantly expressed growth factor may be advantageous for increasing survival rates of AA breast cancer patients. Fortunately, ongoing clinical efforts are currently underway to hinder EGFR expression in TNBCs. A newly developed promising EGFR inhibitor and monoclonal antibody, cetuximab (Erbitux), has stimulated some anti-tumor activity when combined with the chemotherapeutic drug, carboplatin, in a phase 2 clinical trial with advanced TNBC patients (41). Overall response rate and progression-free survival were dramatically improved rendering the targeted drug a potentially new favorable therapeutic alternative for treatment of TNBCs and AA breast cancer patients frequently afflicted with this aggressive breast cancer subtype (41). The accompanying CDx, EGFR immunohistochemical staining, has been approved for clinical practice for colorectal tumors but debates persist about the clinical utility of this assay as cetuximab has only reaped adequate success in tumors harboring wild-type KRAS (15). Therefore, an additional CDx test that assays the status of this response modifier may be necessary in order to enhance the clinical utility of EGFR inhibitors. Hence, proper surveillance of the mutational landscape of each AA breast tumor may be imperative in sufficiently targeting EGFR mutation and other molecular anomalies in AA breast cancer patients.

Thus, through designing therapeutics and their companion assays, it might be possible to significantly reduce the disparities in clinical outcomes among AA and EA breast cancer patients. However, these efforts are still in their premature stages and only a few genetic alterations have received adequate attention. Moreover, there remain countless newly discovered differentially expressed genes and other inherent biological differences, responsible for the increased metastatic risk in AA breast cancer patients, worthy of being targeted.

5. UNDER THE RADAR: OTHER POTENTIAL THERAPEUTIC TARGETS FOR AA BREAST CANCER PATIENTS

As previously discussed, molecular diagnostics has been subtly targeting differentially expressed genes contributing to aggressive-like characteristics demonstrated by AA breast tumors. Yet, with the concept of personalizing medicine for AA breast cancer patients quickly gaining momentum, a vast array of “aggressive” genes and other molecular anomalies linger for consideration as potential clinical therapeutic targets (Table 3). Investigating additional well-documented genetic alterations of AA breast cancer cells for anti-cancer therapeutics is necessary for mitigating breast cancer-related racial disparities in clinical outcomes.

5.1. Genes associated with tumor microenvironment and facilitating metastasis

Intrinsic differences within the tumor microenvironment of ethnically distinct breast tumors have been strongly suggested to serve as key players in driving stark differences in disease course among AA and EA breast cancer patients. Several gene expression microarray studies have consistently reported L-3 phosphoserine phosphatase homolog (PSPHL) as one of the most highly differentially expressed genes of the tumor epithelium and stroma among AA and EA breast tumors (9). This may come as no surprise as PSPHL

Table 3. Potential therapeutic targets and CDx assays that may be beneficial for AA breast cancer patients

Target (s)	Mechanism of facilitating tumor growth	Differential expression in AA	Potential therapeutic (s)	Potential CDx
Tumor microenvironment				
L-3 phosphoserine phosphatase homology (PSPHL)	ECM remodeling; cytokine and growth factor expression interference; reduction of apoptotic activity	Overexpressed	Small-molecule inhibitors that antagonize genes of the tumor epithelium or stroma	IHC assay, gene sequencing
Crystalline beta 2 (CRYBB2)	Suggested to promote tumor growth in AA breast carcinomas	Overexpressed	Small-molecule inhibitors that antagonize genes of the tumor epithelium or stroma	IHC assay, gene sequencing
Sons of sevenless drosophila homolog 1 (SOS1)	Signal transduction, cell growth, cell differentiation, upregulation of RAS/MAPK signaling pathway	Overexpressed	Small-molecule inhibitors that antagonize genes of the tumor epithelium or stroma	IHC assay, gene sequencing
DNA repair pathways				
Partner and localizer of BRCA2 (PALB2)	Works with BRCA2 protein to repair damaged DNA and impede tumor growth	Mutation	PARP1 inhibitors	Gene sequencing
Checkpoint Kinase 2 (CHEK2)	Inhibits cells from undergoing mitosis when DNA damage is present	Mutation	CHEK2 inhibitors, PARP1 inhibitors	Gene sequencing
BRCA1-associated RING domain protein 1 (BARD1)	Works with BRCA1 protein in DNA damage repair and apoptosis intiation	Mutation	Histone deacetylase inhibitors (HDAC inhibitors)	Gene sequencing
Ataxia telangiectasia mutated (ATM)	Recognizes damaged DNA and coordinates DNA damage repair	Mutation	ATM inhibitors, PARP1 inhibitors	Gene sequencing
Phosphatase and tensin homolog (PTEN)	Induces apoptosis and regulates cell growth by interfering with PI3K/AKT pathway	Mutation	PTEN inhibitors, PARP1 inhibitors	Gene sequencing
Tumor protein p53 (TP53)	Regulates cell division by preventing cells with mutated or damaged DNA from dividing and developing into tumors	Mutation	Small-molecule inhibitors that interfere with MDM2-p53 interaction	Gene sequencing
Cell cycle				
Cyclin E	Promotes progression of cell cycle through G1-phase and initiates DNA replication through interacting with cyclin dependent kinase 2 (CDK2)	Overexpressed	CDK2 inhibitors	IHC assay
Cyclin dependent kinase inhibitor 2A (p16)	Inhibits progression of cell cycle from G1-phase to S-phase by phosphorylating Rb protein	Overexpressed	CDK4 inhibitors	IHC assay
Cyclin D1	Promotes progression of cell cycle through G1 phase	Underexpressed	Molecules to induce expression	IHC assay

Contd...

Table 3. *(Continued)*

Target (s)	Mechanism of facilitating tumor growth	Differential expression in AA	Potential therapeutic (s)	Potential CDx
Metastasis-promoting genes				
ATPase, Na+/K+transporting, beta 1 polypeptide (Atp1b1)	Enzyme responsible for regulating electrochemical gradients of Na and K ions across plasma membrane	Higher expression compared to EA	Molecules to reduce expression	IHC assay
Caspase recruitment domain family, member 10 (CARD 10)	Apoptosis signaling through protein-protein hemophilic interactions	Lower expression compared to EA	Molecules to induce higher expression	IHC assay
Kruppel-like factor 4 (KLF4)	Transcription factor that regulates cell proliferation, differentiation, and apoptosis	Lower expression compared to EA	Molecules to induce higher expression	IHC assay
Serine peptidase inhibitor, Kunitz type 2 (Spint2)	Putative tumor suppressor	Lower expression compared to EA	Molecules to induce higher expression	IHC assay
ATP citrate lyase (Acly)	Enzyme responsible for synthesizing cytosolic acetyl-CoA	Higher expression compared to EA	Molecules to reduce expression	IHC assay
Gene polymorphisms				
UDP-glucoronosyltransferase (UGT) 1A1 locus	Regulates glucuronidation chemical reactions for cell metabolism	Variation in TA repeats	Lack of data	Gene sequencing to detect polymorphisms
Mitochondrial DNA G10398A gene	Encodes for subunits of the electron transport chain of the inner mitochondria membrane	Amino acid substitution of A by G	Lack of data	Gene sequencing to detect polymorphisms
Gene copy numbers alterations (CNAs)				
CNAs	Characterize majority of breast cancers	Higher presence in triple negative tumors	Lack of data	Automated systems to detect alterations in gene copy numbers, gene sequencing
Epigenetic modifications				
DNA methylation	Frequent deregulation in breast cancer cells	Hypermethylation	DNA methylation inhibitors	Epigenetic profiling
Organellar distinctions				
Centrosomes	Amplified in breast cancer cells and suggested to serve as principal drivers of breast cancer aggressiveness	Strong association of centrosome amplification in etiology of TNBC	Centrosome declustering agents, PARP1 inhibitors, HSET inhibitors	Lack of data; methods of precise detection of extent and magnitude of centrosome amplification needed

plays a chief role in ECM remodeling, driving cytokine and growth factor expression, and diminution of apoptotic activity (43). Substantiated to influence the aggressive phenotypes displayed by AA breast tumors, it is believed to be a promising therapeutic target for AAs (42, 43). The crystalline beta 2 (CRYBB2) gene follows close behind PSPHL as one of the most highly differentially expressed genes of the tumor epithelium among AA and EA breast tumors (9). An essential protein embedded in the eye lens of vertebrates, aberrant expression of this protein in the breast has been linked with augmented tumor growth in rat models and highly suggested to bolster growth of AA breast carcinomas (43). These data suggest CRYBB2 as a valuable target of interest for anti-cancer therapeutics for AA breast cancer patients. Another tumor stroma gene worthy of speculation, sons of sevenless drosophila homolog 1 (SOS1), has been generating buzz as a prominent genetic alteration in AA

breast tumors (43). SOS1, a member of the RAS family, is intricately involved in signal transduction, cell growth, cell differentiation, and upregulation of the RAS/MAPK signaling pathway (43). With gene expression microarray studies corroborating distinct differences in expression profiles of this gene among AA and EA breast tumors, SOS1 may deserve attention as a potential therapeutic target for AA breast cancer patients. With PSPHL, CRYBB2, and SOS1 bearing strong implications in tumor differentiation, invasion, and metastasis, Martin *et al*, consulted a Connectivity Map database to pinpoint small molecules that may antagonize differentially expressed genes of the microdissected tumor epithelium and stroma of AA and EA breast tumors (9, 43). Identification of these antagonists prompt further exploration into their development as potential targeted therapeutics for AA breast cancer patients along with validated CDx that can identify patients that will benefit from them in the clinic.

Targeting genes well documented to serve as critical drivers of cancer metastasis is an additional pragmatic route that merits much attention in devising effective anti-cancer therapeutic agents for AA breast cancer patients. Through comparative *in vitro* experiments, a recent study identified the metastatic genes, Atp1b1, CARD 10, KLF4, Sprint2, and Acly to be differentially expressed between AA and EA breast cancer cell lines suggesting a causative role of these genes in the aggressive tumor phenotypes exhibited in AA breast cancer patients (6). Thus, differentially expressed metastatic genes also deserve attention as potential therapeutic targets for AA breast cancer patients. Development of inhibition mechanisms of these crucial drivers of tumor progression and clinically applicable methods of assessing their aberrant expression, could prove to be useful for AA breast cancer patients.

5.2. Genes associated with maintenance of genomic integrity

As previously mentioned, a high frequency of mutations in the BRCA1/2 genes predisposing AA women to aggressive breast tumor phenotypes has become a strong point of interest for anti-cancer therapeutics as targeting other breast cancer susceptibility genes may provide another reasonable route to thwart the susceptibility of AA women to acquiring aggressive breast cancer. A recent study has employed DNA sequencing technologies to cost-effectively screen multiple genes simultaneously for mutations in all identified breast cancer susceptibility genes in AA breast cancer patients. In addition to BRCA1 and BRCA2, the assay detected notable alterations in the breast cancer predisposition genes, PALB2, CHEK2, BARD1, ATM, PTEN, and p53 in AA breast cancer patients (29). These genes normally play vital roles in maintaining genomic integrity and response to DNA damage. This data prompts incentives for the development of more novel therapies targeting anomalies in DNA repair pathways that may be boosting the risk of AA women acquiring life-threatening breast cancer subtypes. Routine clinical genetic testing may be more beneficial for AA breast cancer patients if they encompass these additional breast cancer susceptibility genes suggested to be frequently mutated in AA patients. These individuals may be sensitive to PARP1 inhibitors as a viable therapeutic approach (29). Hence, a more robust effort can be adopted in clinics by targeting multiple anomalies in DNA repair pathways, although more genomic testing for these molecular alterations may come at a very high cost posing a major barrier to incorporating this strategy (29).

5.3. Genes associated with cell cycle machinery

Aberrations in cell cycle progression have long been recognized to contribute to the more aggressive growth of AA breast tumors and studies have revealed differentially expressed cell cycle proteins among AA and EA breast tumors. As previously mentioned, the well-recognized cell cycle protein, p53, is already in the early stages of being targeted for anti-cancer therapeutics. In addition to the overexpression of p53, elevated expression levels of cyclin E and p16 as well as low expression levels of cyclin D1 have been reported in estrogen receptor (ER)–negative, high grade AA breast tumors of higher mitotic index in young women (44). Hence, aberrations in cell cycle gene expression may be critical drivers of disparate clinical features and behavior among AA and EA breast tumors, and thus, warrant considerable attention in efforts to eliminate breast cancer health disparities. Anti-mitotic drugs however have performed dismally in clinical trials highlighting the need for researchers to revisit, explore and understand more deeply subtle differences in tumor biology and *in vivo* growth kinetics between AA and EA breast tumors (24). The identification and validation of novel and early biomarkers that foretell aggressive disease course in AAs and shed light on the interplay of ancestry in determining tumor phenotypes is thus direly needed.

5.4. Spotlight on genetic polymorphisms, copy number alterations, and epigenetic modifications

Although the focus of biological disparities among racially distinct breast tumors have largely been on gene mutations, differences in inherent gene polymorphisms between AA and EA breast tumors may deserve some light as potential drivers of disparities in clinicopathological features. Several studies have reported divergence in particular gene sequences among AA breast tumors potentially underlying the increased risk of AA to developing invasive breast cancers in comparison to EA women. Guillemette, *et al*, identified variations in the number of TA repeats within the UDP-glucoronosyltransferase (UGT) 1A1 locus in women of African ancestry positively correlating with their susceptibility to invasive breast cancer, earlier onset,

and acquirement of ER-negative breast tumors through attenuating the effect of endogenous hormones (45). Canter, *et al*, discovered polymorphic variation in the mitochondrial DNA G10398A gene in AA is strongly associated with their increased risk of acquiring invasive breast cancer (10). In conjunction with enhanced metastatic risk, genetic polymorphisms can influence drug metabolism and sensitivity suggesting a strong role in disparities in response to anticancer therapeutics among AA and EA breast cancer patients (46). Advances in molecular diagnostic testing for gene polymorphisms involved in the etiology of each patient's cancer and those implicated in poor prognosis could yield new therapeutic strategies by further refining the blueprint for personalizing therapy for AA patients and improving their outcomes (46).

Another potential molecular distinction that has received less attention than warranted, are genomic copy number alterations (CNA). CNAs characterize most breast cancers and have been found to be significantly more present in TNBCs compared to non-triple negative breast tumors implying a higher degree of genomic instability in TNBC (47, 48). Bergamaschi *et al*, discovered CNAs correlated with aggressive clinicopathological features of breast cancer such as high tumor grade, ER-negativity, basal-like subtypes, p53 mutations, and poor overall survival (49). With AA breast cancer patients exhibiting a higher frequency of analogous aggressive tumor characteristics, CNAs may require some further investigation as a source of differences in pathology among AA and CA breast cancer patients and a potential basis for augmented clinical prognostication (49).

In addition to the somatic alterations in gene expression detailed above, studies have also reported significant differences in DNA methylation patterns among AA and EA breast tumors with a higher frequency of hypermethylation observed in tumor suppressor genes in AA compared to EA patients (8). Furthermore, hypermethylation profiles significantly varied among ER-, PR-, and women of younger age in AA compared to EA patients. (50). These findings further illuminate the basis of tumor biological differences between AA and EA breast cancer patients. Long acknowledged to bear therapeutic implications in cancer, epigenetic modifications may presumably be targeted by therapeutics for AA breast cancer patients and may serve as a preemptive alternative measure in preventing aberrant expression of genes conferring them to a poor clinical prognoses. Fortunately, there are current ongoing efforts to pioneer novel DNA methylation inhibitors such as 5-aza-2' deoxycytidine (5-Aza), which operate by inhibiting DNA methyltransferases (DNMTs) to stimulate DNA hypomethylation and subsequently reexpression of the tumor suppressor gene (51). Rapid development of these new therapeutic modalities urges the assimilation of epigenetic profiling into clinical practice for selection of AA breast cancer patients who might benefit from these agents. Identification of a robust panel of DNA methylation biomarkers is presently a strong focus of interest in research for the development of CDx to identify patients who will potentially be sensitive to DNA methylation inhibitors (52).

5.5. Delving deeper into disparity: Time to pay heed to organelle–level differences

In addition to the molecular-level disparities that have captured a spotlight in the cancer health disparity community, recently discovered disparities at the organellar level are slowly creating a stir as attractive therapeutic targets for AA breast cancer patients. Centrosome amplification is a potent driver of chromosomal instability and underlies acquisition of aggressive phenotypes (53, 54). Pannu *et al*, further corroborated these assertions recently by reporting that TNBCs harbored much higher levels of centrosome amplification than non-triple negative breast tumor subtypes (55). With a three-fold higher incidence of TNBC among AA compared to EA breast cancer patients (56), centrosome amplification theoretically serves as a viable candidate for therapeutic intervention that might particularly benefit AA breast cancer patients. Since cancer cells employ centrosome clustering mechanisms to avert mitotic catastrophe and maintain an optimal level of persistent aneuploidy that fuels tumor progression (57, 58), centrosome declustering has emerged as a compelling therapeutic strategy to selectively target cancer cells harboring excess centrosomes (59). Centrosome declustering drugs such as griseofulvin, noscapine and its derivatives, and inhibitors of centrosome clustering mechanisms bear strong clinical implications for AA breast cancer patients frequently afflicted with TNBC. Recently, two promising inhibitors (CW069 and AZ82) have been developed for centrosome clustering protein HSET, and are commercially available from Pfizer and Astra Zeneca (60, 61). These HSET inhibitors could therefore bear significant therapeutic value for AA breast cancer patients. Successful use of these novel therapeutic agents for AA patients would require development and validation of reliable methods to assess centrosomal profiles, and accurate quantitation of the prevalence and severity of centrosomal aberrations in clinical samples.

The above mentioned genetic alterations and organellar disparities are just a few of the countless reported inherent and potentially actionable biological differences suggested to be conferring AA breast tumors with aggressive clinical features. Individualized treatment regimens involving targeted therapies based upon the specific biomarker profiles of AA breast cancer patients (as uncovered by batteries of CDx) will likely play a key role in improving outcome for AAs and alleviating ethnic health disparity. However, further research is needed to

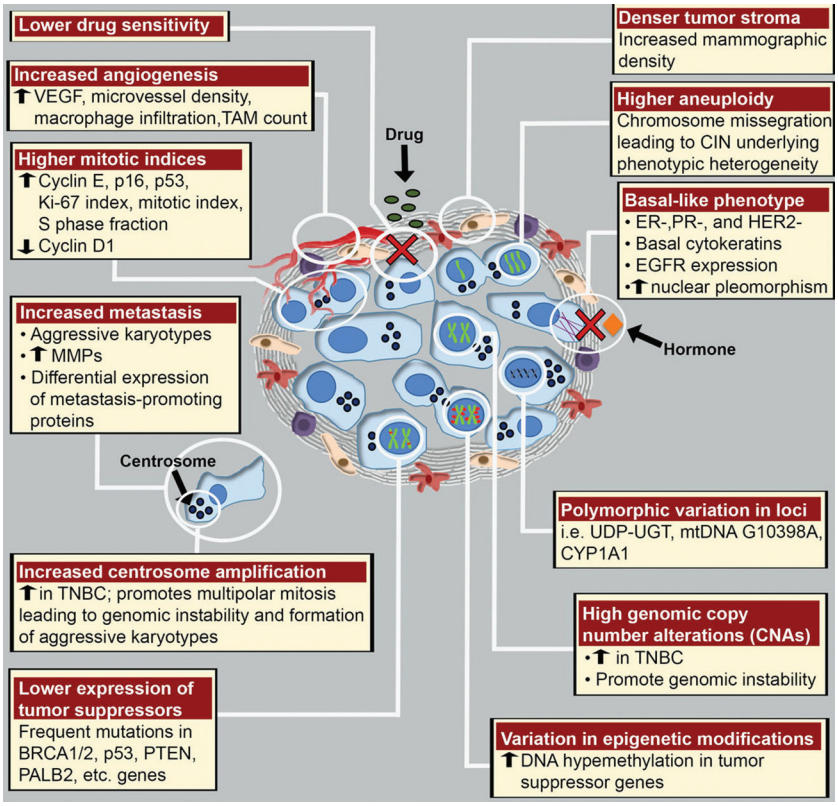

Figure 1. Distinctions in inherent tumor biological characteristics among AA and EA breast tumors. Illustration of the differences in the biological tumor make-up of AA and EA breast tumors that promote inequities in tumorigenesis, survival, and treatment response among the ethnic groups. These inherent biological disparities include distinctions in tumor architecture and stroma, tumor suppressor gene expression, cell cycle protein expression, genomic copy numbers, genetic polymorphisms, epigenetic modifications, mitotic indices, cellular aberrations, organellar aberrations, angiogenesis, and phenotypic heterogeneity. These variations in tumor biology between the ethnicities have been strongly suggested to underlie the ethnic disparity in breast cancer mortality and warrant consideration as viable therapeutic targets.

unlock more hidden biological distinctions among AA and EA breast tumors.

6. THE LONG ROAD AHEAD: CHALLENGES IN TAILORING TREATMENTS FOR AA BREAST CANCER PATIENTS

The road to translating recent findings related to molecular and organelle-level differences between AA and EA breast tumors into valid clinical diagnostic and therapeutic approaches, is beset with a litany of challenges, both biological (Figure 1) and non-biological, that need to be addressed. The clinically influential role of CDx assays requires them to bear a high tenor of analytical and clinical validity affirmed through extensive pre-clinical and clinical testing. Strict regulatory CDx standards and guidelines need to be implemented by the FDA and other regulatory institutions to ensure these measures are adequately met (13). More effective monitoring of co-developed CDx requires the establishment of clearer criteria by the FDA for regulating laboratory-developed *in vitro* diagnostic tests, the charting of unambiguous regulatory and policy guidelines for bringing more innovative CDx to the market, and the garnering of more solid evidence of success rates of approved drugs or their CDx assays (62, 63, 64). Inconsistencies in CDx assay measures of specific proteins or genetic mutations in the clinic may lead to undesirable false positives or negatives or subject patients to harm and toxic side effects, which may comprise grave impediments to the development of safe targeted therapeutics for AA breast cancer patients (13) (Figure 2).

Another oft-neglected complication related to CDx assays relates to the use of primary tumor tissue samples in clinical studies aimed at biomarker validation. For AA patients with metastatic breast tumors, clinical CDx tests are often used to select treatments for a disease that is at a much later stage compared to the primary tumor tissue which is evaluated for biomarker status (65). With the high risk of new molecular modifications accruing during tumor evolution, and the difficulty involved in obtaining samples from metastatic sites for determining best treatment options, this drawback could potentially undermine the strength of the predictive CDx assay. Additional hurdles to the

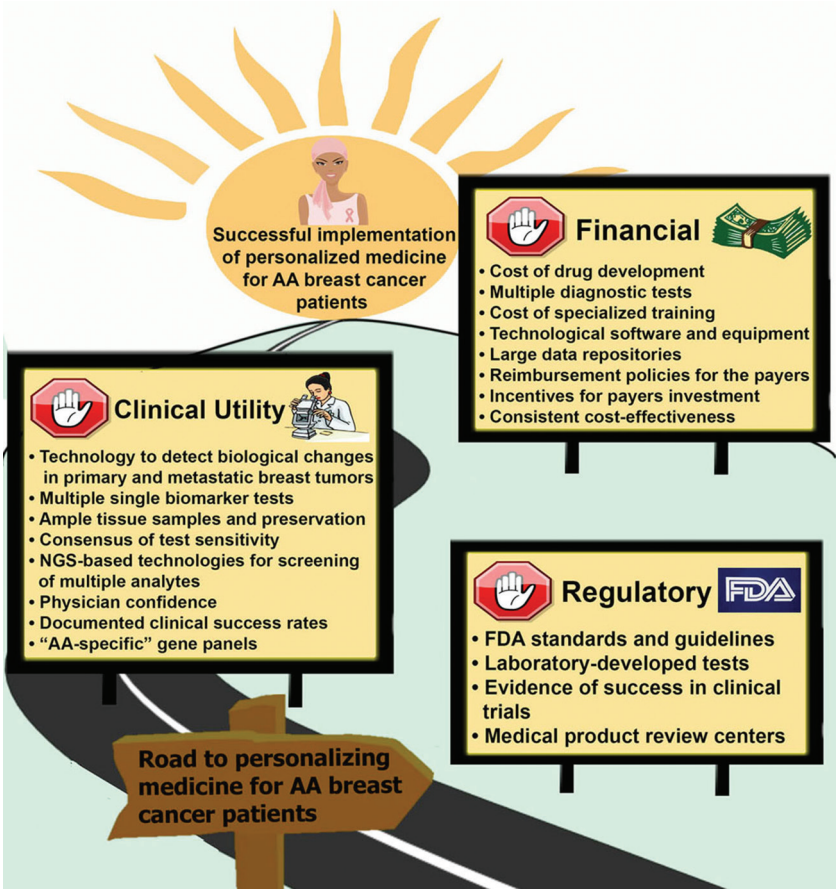

Figure 2. Challenges in personalizing treatments for AA breast cancer patients in the clinic. Depiction of potential obstacles that may complicate the process of tailoring medicine for AA breast cancer patients in the clinic. Personalizing medicine for AA breast cancer patients will entail translating inherent tumor biological characteristics that contribute to their poorer clinical outcomes into CDx assays and targeted therapeutics for routine clinical use. One of the major challenges that need to be addressed in this regard include those related to institution of clear regulatory criteria and guidelines for review and approval of CDx assays. Meeting these requirements will entail intense scrutiny according to FDA standards and guidelines, success in LDT and clinical trials, and sanction from medical product review centers. Another looming obstacle includes rigorously-validated standards for establishing clinical utility of both the drug and the CDx. These strict criteria will encompass well-established test sensitivity levels, validated multiple biomarker assays, proficient technology for optimum detection precision, and well-documented high clinical success rates. The biggest challenge will involve prohibitive costs associated with gene expression-based CDx, the requirement of specialized training and expertise to run and interpret such tests, development of technologies and equipment for running the tests, and getting these tests reimbursed for patients and clinics in order to make this approach cost-effective.

application of personalized medicine for AA breast cancer patients in the clinic include (a) detection of a single therapeutic target, which is often insufficient to properly guide treatment selection due to the existence of additional mutations that might modify drug response or interfere with drug efficacy (66), (b) lack of consensus on reliable drug sensitivity values once the gene mutation of interest is detected and on the percentage of the lesion that needs to express this mutation in order to generate a viable response to the therapeutic drug (65), and (c) intratumoral heterogeneity in biomarkers and the existence of distinct molecular alterations and genomic rearrangements in different parts of the tumor, that have generated cracks in the classic "one test, one target" paradigm of personalized medicine (67). In order to address these complexities, savvy alternative approaches to diagnostic testing of multiple markers are rapidly revolutionizing the framework of personalized medicine (67). The simultaneous identification of all genetic aberrations via NGS, or the screening of multiple analytes simultaneously via high-throughput testing mass spectrometry (MS) proteomics are driving a shift from the inefficient "one test, one target" presently implemented approach of personalized medicine to a more pragmatic "one test, multiple targets" model as illustrated in Figure 3.

The shift of personalized medicine from a "one size fits all" archetype to a more tailored therapeutic stratagem is theorized to potentially curtail the astronomical health care costs presently plaguing the healthcare industry (68). Stratification of AA breast

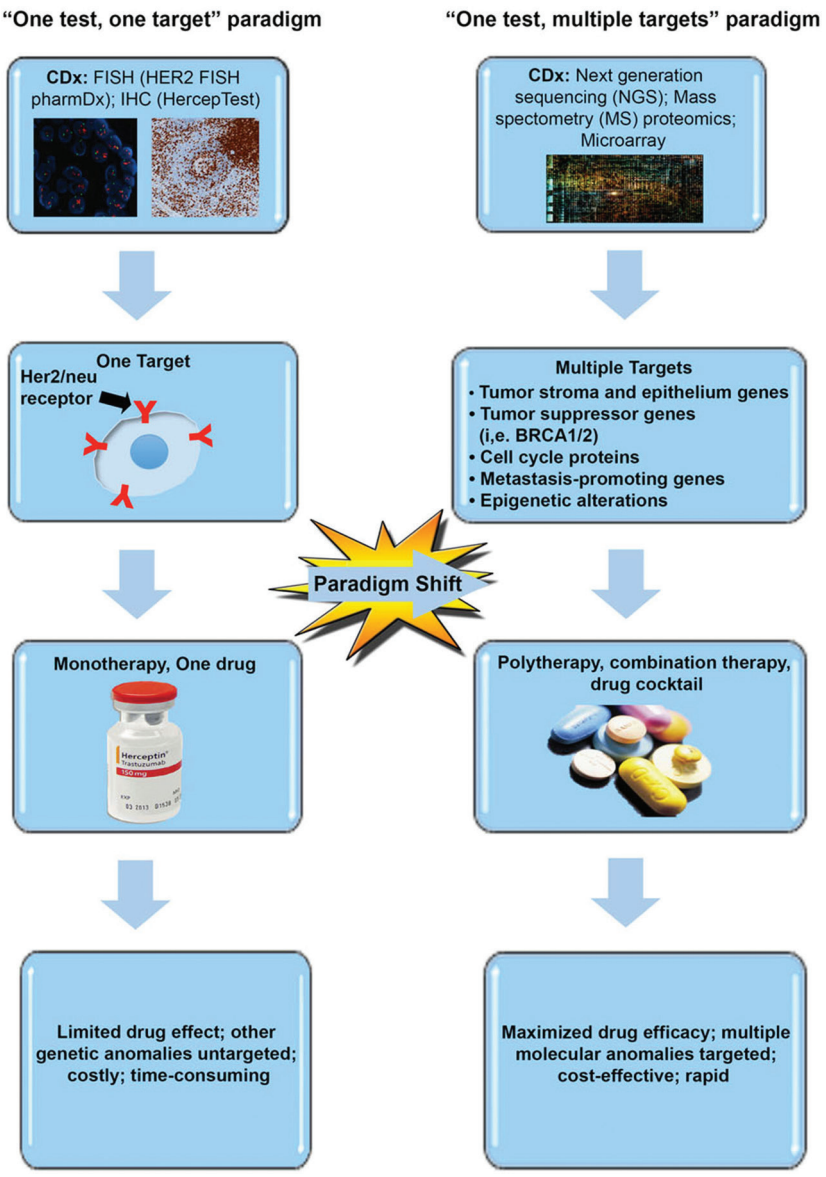

Figure 3. Paradigm shift in personalized medicine from a "One test, One target" model to a "One test, Multiple targets" model. Schematic representation of the anticipated change in landscape of personalized medicine in the clinic from employing low-throughput diagnostic testing platforms such as IHC and FISH that identify single therapeutic targets to exploiting advanced NGS-based technology and mass spectrometry that screen for multiple analytes simultaneously. This paradigm shift in standard clinical diagnostic practices may be advantageous for AA breast cancer patients by identifying the manifold of inherent tumor molecular distinctions underpinning their poorer clinical outcomes than EA breast cancer patients. This shift may encourage the implementation of combination or polytherapeutic approaches that target multiple molecular anomalies in AA breast cancer patients, theoretically augmenting their clinical response.

cancer patients for customization of treatments in the clinic may contribute significantly to this decline in health care expenditure. Although drug efficacy rates are likely to be boosted by the utilization of robust CDx assays, the transition to CDx-dependent therapy selection may breed disincentives for pharmaceutical industries and the payers, due to the spike in cost of drug and assay development (68). Moreover, NGS and other forms of multiple diagnostic testing in clinics may even increase health care costs as the number of diagnostic exams and therapeutic drugs surge. Additional economic challenges that may inevitably surface from personalizing medicine for AA breast cancer patients include lack of reimbursement for the payers to adequately supplement development of high-value tests (69). Incentives for payers to invest in the development of targeted therapeutics for AA breast cancer patients may also be weak due to documented failures or low response

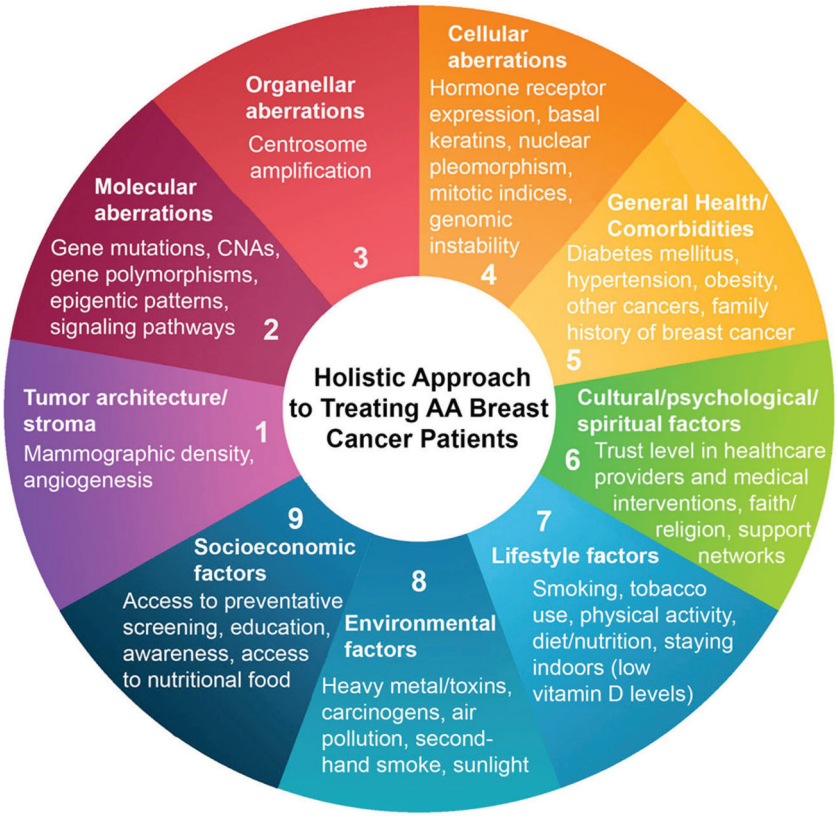

Figure 4. "Holistic" approach of personalizing medicine for AA breast cancer patients encompassing all established biological and non-biological disparities. The successful implementation of personalized medicine for AA breast cancer patients will require scrutiny of both biological disparities (1-4) comprising molecular, organellar, and cellular aberrations, tumor architecture, and comorbidities as well as non-biological disparities (5-9) including cultural/spiritual, environmental, socioeconomic, and lifestyle influences. Targeting all reported biological and non-biological factors will theoretically elicit a more robust pathological clinical response for AA breast cancer patients.

rates of current tailored medicines utilized in the clinic, physician skepticism in adopting tailored therapeutic approaches in lieu of their traditional treatment recommendations, and lack of evidence supporting satisfactory competency of targeted drugs in the clinic (70). Furthermore, inconsistencies in cost-effectiveness of presently implemented CDx tests in clinics may beget more uncertainty in the payers' mind as to whether subscribing to targeted therapies for AA breast cancer patients will be cost-beneficial for them. For example, Herceptin has been estimated to minimize costs as it affords the avoidance of employing expensive therapies in clinics (71). Conversely, screening for BRCA1 is only cost-effective when performed on patients with a family history of the familial gene mutation (71). These contradictions may prevent investment in targeted anti-cancer therapeutics for AA breast cancer patients. While the above-mentioned obstacles to the implementation of personalized medicine for AA breast cancer patients might appear to be insurmountable problems, it is possible that they are nothing more than opportunities for scientific innovation and bold enterprise aimed at finding means to reduce racial disparity.

7. DIFFERENT STROKES FOR DIFFERENT FOLKS: PERSONALIZED MEDICINE MAY DIMINISH THE RACIAL DISPARITY IN BREAST CANCER OUTCOMES

If the bumps in the road to personalizing medicine for AA breast cancer patients in the clinic can be smoothed out, and CDx assays that evaluate key drivers of aggressive disease course in AA breast cancer patients can be developed alongside novel and efficacious therapeutics, improvements in breast cancer outcomes for AAs are indeed foreseeable. The dawn of genetic, epigenetic and organellar profiling prior to guided clinical therapy can potentially enhance the accuracy of prognosticating the disease course and predicting responsiveness to drugs. This strategy could enable more accurate identification of AA patients in need of more aggressive treatments, while sparing AAs with less aggressive disease the undesirable side effects of such treatments. As discussed earlier, although implementation of NGS may revolutionize clinical oncologic diagnostic practices by detecting mutations in numerous genes simultaneously, it would

do so at a significant cost. Centrosome status profiling would provide a less expensive and more facile means to target novel pathways very selectively and effectively in cancer cells. The use of CDx assays will also facilitate clinical trial design so that patients for the trial are selected such that they are more likely to respond to the drug in question; such trials will therefore have a smaller number of subjects, which has a positive effect on the resources and time spent on clinical development while enhancing chances of success (13). With an astounding array of distinctions that are now recognized between AA and EA breast carcinomas, devising "African ancestry-focused" gene panels and methodologies for quantitating centrosome amplification would indubitably go a long way in improving outcomes for AAs. Successful implementation of ethnicity-specific gene panels in clinics, may require incorporation of ancestry typing for individuals of mixed ethnicity. More importantly, studies that delineate the minimum proportion of African ancestry required for the assay and therapy to be of significant benefit to a given individual need to be carried out, to enhance precision of biomarker and assay selection for individual patients.

Personalized oncology today is however, far too "gene-centered" and therefore, limited in its reach. The successful exploitation of personalized medicine requires a more "holistic" approach encompassing both biological differences including the previously mentioned organelle-level disparities, age at menarche, and comorbid conditions, as well as non-biological disparities such as socioeconomic disproportions, environmental influences, and lifestyle factors, as depicted in Figure 4. Each of these layers, comprised of biological and non-biological components, are concertedly contributing to inequalities in clinical outcomes among AA and EA breast cancer patients and warrant equivalent scrutiny. Integration of these layers into a robust individualized therapeutic approach for each AA breast cancer patient involving precisely validated methods of assessments partnered with companion targeted therapies will champion a future of pivotal breakthroughs aimed at eliminating breast cancer health disparity.

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