Triple Negative Breast Cancer

A Paradox or Pandora's Box

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This is to acknowledge that Barbara Haley, M.D. has disclosed financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Barbara Haley will not be discussing off-label uses in his/her presentation.

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INTRODUCTION

In 2008 an estimated 1.3 million women world-wide were diagnosed with breast cancer and of these about 180,000 new breast cancer cases were in the US. Statistics give US women a 1 in 8 probability of developing breast cancer in her lifetime.

The projected 2008 death rates from breast cancer world-wide were 465,000 deaths with 40,000 breast cancer deaths in US women making breast cancer the second leading cause of cancer deaths in women after lung cancer. [1]

Despite grim statistics, breast cancer mortality has declined due to earlier and improved screening, better surgical and radiation techniques and the benefit from adjuvant systemic therapy.

ADJUVANT SYSTEMIC THERAPY

Traditionally, to choose adjuvant systemic therapy, a practicing oncologist relies heavily on clinical and pathologic features of the tumor. These conventional features include tumor histology and morphology (infiltrating ductal, lobular, medullary, etc…) along with tumor size, nodal status, grade, and immunohistochemical assays that define the tumor cell molecular markers such as the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression. These factors have recognized prognostic and predictive value and serve as a basis for choosing systemic therapy options.

For patients with hormone receptor positive tumors (ER+/PR+ or ER+/PR- or ER-/PR+), a hormonal therapy (tamoxifen or an aromatase inhibitor) is appropriate.. Since about 70% of all breast cancers have some form of hormone receptor expression, a hormonal therapy will be involved in a large portion of breast cancer treatment. For HER2 positive patients, comprising about 15% of all breast cancers, a biologic targeted therapy against HER2 with either trastuzumab monoclonal antibody or a small molecule tyrosine kinase inhibitor lapitinib will be added into the treatment regimen.

However, there is a third group of breast cancer patients that don't belong in either of these categories. This group lacks both hormonal receptors (ER and PR negative) and lacks HER2 expression and is termed Triple Negative (TN) breast cancer (i.e. lacks ER, PR and HER2). The TN group is 8 to 20% of all breast cancer and is numerically not a negligible number of patients. [2] Unlike the hormone receptor positive and HER2 positive breast cancer groups, TN tumors lack a specific molecular target and have only non-targeted chemotherapeutic approaches for treatment.

IMPORTANCE OF THE TRIPLE NEGATIVE BREAST CANCER (TNBC) GROUP

CLINICAL IMPACT:

Globally TN breast cancer can be a devastating disease as it tends to occur in younger women, is characterized by early relapse and rapid emergence of resistance to further therapy, is associated with the worst breast cancer prognosis and causes a disproportionate number of breast cancer deaths compared to the other types of breast cancer.[3]

SCIENTIFIC AND THERAPEUTIC IMPACT:

Until a few years ago, Triple Negative breast cancer was not recognized as a distinct clinical and molecular entity. Recent sophisticated research techniques have identified that breast cancer is not a single disease but is heterogeneous with unique subgroups like the TN.

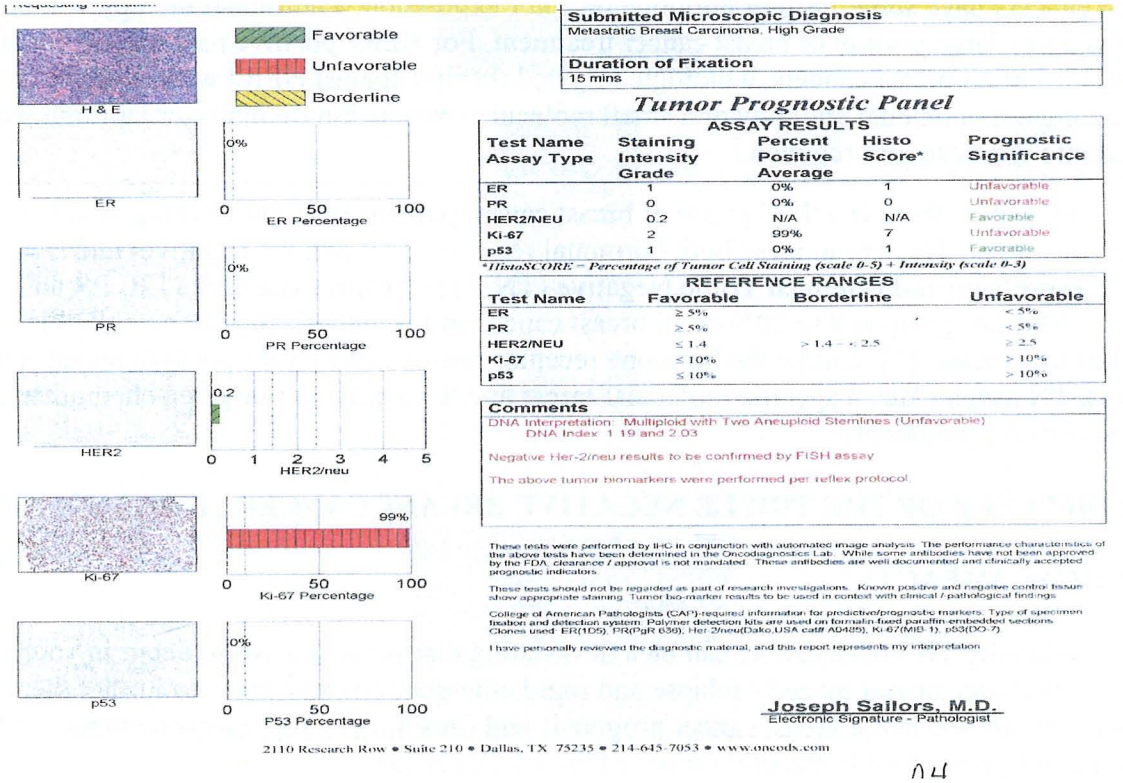
By characterizing the complex molecular and biologic aspects of TN disease, we have opened a scientific window and ushered in an appreciation of how tumor molecular variability will determine the future research and development of novel therapeutic approaches intending to improve clinical disease outcome.

CLINICAL CASE

41 year old African-American female with negative screening mammograms 4 months prior to self palpation of a right breast mass

- repeat mammogram and sonogram confirm a 5 cm spiculated mass at the 6 o'clock position in the right breast
- clinical exam confirms a 6 cm mass without erythema, nipple or dermal invasion and no palpable axillary or supraclavicular nodes with the rest of the physical exam negative
- core biopsy shows infiltrating ductal carcinoma, Grade III, ER/PR and HER2 negative

TUMOR IMMUNOHISTOCHEMISTRY PROFILE



Submitted Microscopic Diagnosis
Metastatic Breast Carcinoma, High Grade

Duration of Fixation
15 mins

Tumor Prognostic Panel

ASSAY RESULTS

Test Name Assay Type	Staining Intensity Grade	Percent Positive Average	Histo Score*	Prognostic Significance
ER	1	0%	1	Unfavorable
PR	0	0%	0	Unfavorable
HER2/NEU	0.2	N/A	N/A	Favorable
Ki-67	2	99%	7	Unfavorable
p53	1	0%	1	Favorable

**HistoSCORE = Percentage of Tumor Cell Staining (scale 0-5) + Intensity (scale 0-3)*

REFERENCE RANGES

Test Name	Favorable	Borderline	Unfavorable
ER	≥ 5%		< 5%
PR	≥ 5%		< 5%
HER2/NEU	≤ 1.4	> 1.4 – < 2.5	≥ 2.5
Ki-67	≤ 10%		> 10%
p53	≤ 10%		> 10%

Comments
DNA Interpretation: Multiploid with Two Aneuploid Stemlines (Unfavorable)
DNA Index: 1.19 and 2.03

Negative Her-2/neu results to be confirmed by FISH assay

The above tumor biomarkers were performed per reflex protocol.

These tests were performed by IHC in conjunction with automated image analysis. The performance characteristics of the above tests have been determined in the Oncodiagnostics Lab. While some antibodies have not been approved by the FDA, clearance / approval is not mandated. These antibodies are well documented and clinically accepted prognostic indicators.

These tests should not be regarded as part of research investigations. Known positive and negative control tissue show appropriate staining. Tumor bio-marker results to be used in context with clinical / pathological findings.

College of American Pathologists (CAP)-required information for predictive/prognostic markers. Type of specimen fixation and detection system: Polymer detection kits are used on formalin-fixed paraffin-embedded sections. Clones used: ER(1D5), PR(PgR 636), Her 2/neu(Dako,USA cat# A0485), Ki-67(MIB-1), p53(DO-7).

I have personally reviewed the diagnostic material, and this report represents my interpretation.

Joseph Sailors, M.D.
Electronic Signature - Pathologist

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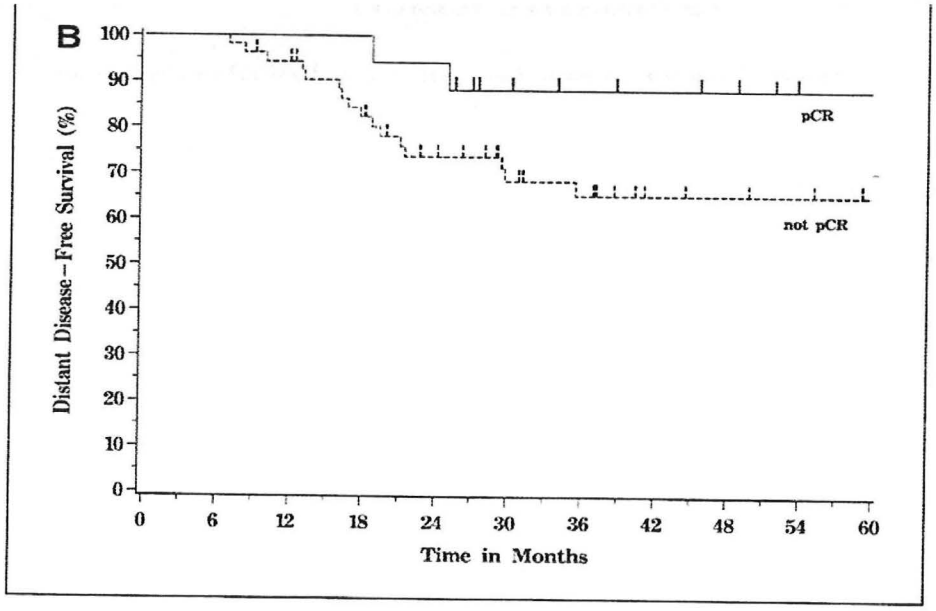
CLINICAL CASE

- Treatment Options: mastectomy with sentinel lymph node dissection or neo-adjuvant induction chemotherapy to reduce tumor size for possible breast preservation
- Treatment – Neo-adjuvant dose dense (DD) ACX4 (doxorubicin and cyclophosphamide every 2 weeks IV) followed by 4 DD paclitaxel
- Response – clinical tumor shrinkage with residual palpable 2.5 cm mass at 16 weeks therapy
- Surgery – partial mastectomy and sentinel node dissection with 4.3 cm residual invasive carcinoma and negative sentinel nodes and clear margins
- Radiation – whole breast radiation 6 ½ weeks
- Follow-up – clinical exams every 3 months and no hormonal adjuvant or further chemotherapy planned
- Prognosis – guarded when a pathologic complete response (pCR) is not achieved at completion of induction chemotherapy

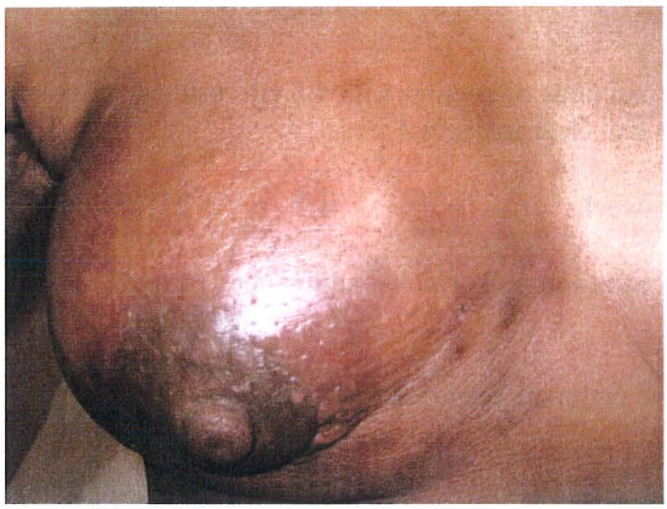
RELAPSE CURVES IN PATIENTS WITH TRIPLE NEGATIVE CANCERS AFTER NEOADJUVANT CHEMOTHERAPY



Carey, L. A. et al. Clin Cancer Res 2007;13:2329-2334

CLINICAL CASE

- Seven months after therapy completion the patient complains of a painful right breast and a skin rash
- The clinical exam reveals palpable and enlarged bilateral axillary lymph nodes and a palpable right supraclavicular node and an abnormal right breast with dermal infiltration, tumor skin nodules and nipple invasion as depicted in the photograph



IDENTIFICATION OF BREAST CANCER SUBGROUPS (INCLUDING BASAL-LIKE BREAST CANCER AND THE TRIPLE NEGATIVE SUBSET)

Until the early 1990's, the study of tumor gene expression was limited to single gene analysis by RNA or Northern Blot analysis. By the mid 1990's, the development of microarray (MA) methodology and sophisticated analytical methods along with high throughput technology allowed the assay of hundreds to thousands of genes at one time creating a molecular signature or fingerprint of an individual tumor. [4-6] A gene expression technique or microarray profile allowed comparison of molecular differences between multiple individual patients with either similar or different clinical and tumor characteristics.

Beginning in 2000, a series of landmark studies were presented by Sorlie and Perou. Using microarray and cDNA methodology, they studied 40 breast cancer samples and 20 matched pairs of tumors before and after doxorubicin treatment and identified 496 genes (intrinsic gene set) chosen on differences on expression between sporadic and paired samples. [7] Using the intrinsic gene set and an unsupervised and hierarchical clustering analysis, they segregated the tumors into 4 major subgroups:

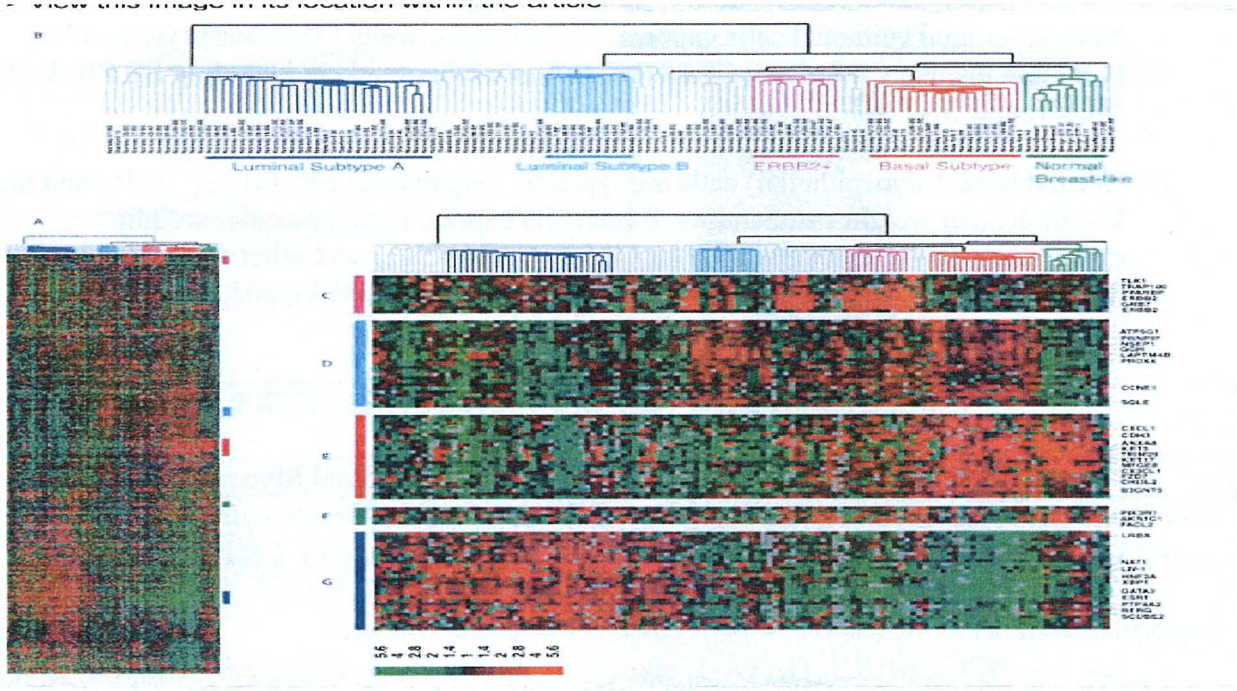
1. Luminal cell-like group -- express the estrogen receptor
2. Basal cell-like group – lacking ER expression
3. HER2 positive group – have HER2 expression
4. Normal breast- like group – more like the basal group and only weak expression of luminal group genes

In a follow up study with 38 additional cancer specimens, the same investigators confirmed the 4 subgroups but separated the Luminal group into Luminal A and Luminal B based on gene expression differences for the estrogen receptor and the GATA-3 gene with Luminal A having the highest estrogen receptor expression and low genomic grade and Luminal B low to moderate expression for the estrogen receptor and higher genomic grade. [8] Thus, there are 5 cancer subgroups: Luminal A, Luminal B, HER2 positive, Basal-like and Normal-like breast cancer.

Further studies revealed a prognostic significance to the subgroups based on significant differences between the groups with Basal-like breast cancer (BLBC) and HER2 positive groups associated with the shortest overall and relapse free survival and the Luminal group particularly Luminal A having the best prognosis. Independent work by other investigators using different tumor specimens and variations of the intrinsic gene set and different array platforms have confirmed the existence of the 5 subgroups and their prognostic association. [9-11]

MICROARRAY HEAT MAP

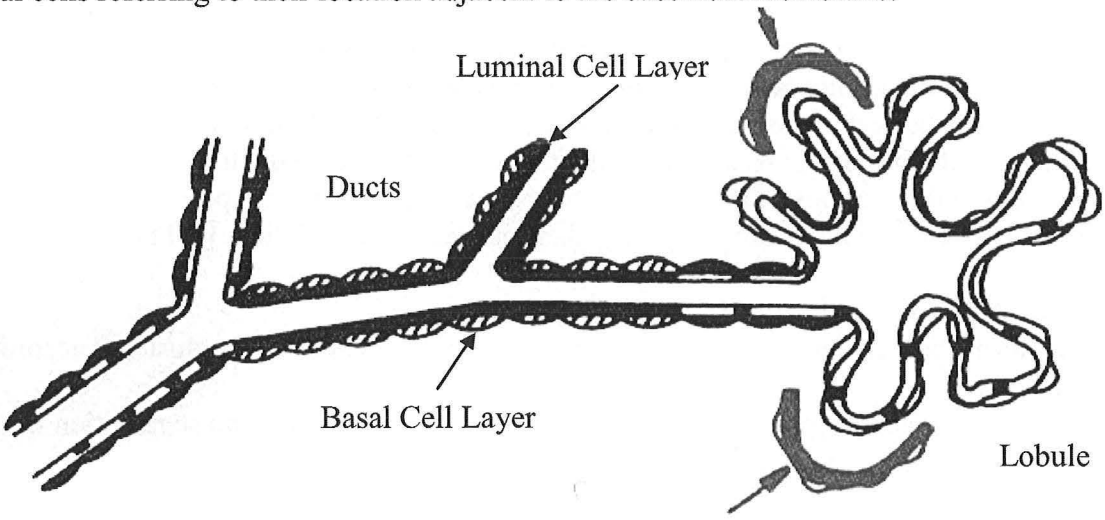
- This illustration contains in A heat map of individual patient samples clustered according to gene sets.
- In B there is a dendrogram showing hierarchical clusters of genes and segregation into the 5 breast cancer subgroups.
- Genes are listed along the vertical axes by individual blocks.
- Red represents up-regulated gene expression
- Green represents relative down-regulation of gene expression
- The overview reflects the heterogeneity of breast cancer and the shared gene features within molecular subgroups.



Sorlie, T. et al, PNAS (USA 2003) 100:8418-23

NORMAL MAMMARY GLAND STRUCTURE – UNDERSTANDING THE TERMINOLOGY

Normal human mammary gland structure consists of ducts and lobules lined by an inner layer, the luminal cells, then the basement membrane and an outer cell layer adjacent to the basement membrane. The outer layer consists of cells that have features of both epithelial and smooth muscle cells and hence termed myoepithelial cells. Myoepithelial cells are also called basal cells referring to their location adjacent to the basement membrane.



BASAL AND LUMINAL IMMUNOPHENOTYPES

The immunophenotype of the 2 cell layers differ:

1. Normal luminal epithelial cells express low molecular weight (luminal) cytokeratins (CK) that include CK7, CK8, CK18 and multiple other markers including ER,PR, BCL2, MUC1, and GATA-3.

2. Normal basal (myoepithelial) cells are typically negative for ER, PR and HER2 and the low molecular weight cytokeratins. Basal cells express high molecular weight cytokeratins (basal cytokeratins) like CK5/6, CK14, CK17 and other molecular markers like EGFR, c-kit, caveolin1, S-100, smooth muscle actin (SMA), p63, P-cadherin, calponin, etc… [2, 12, 13]

IMMUNOPHENOTYPIC MOLECULAR MARKERS	
Normal Luminal Epithelial Cells	Normal Basal Myoepithelial Cells
Low molecular weight cytokeratins • CK7, CK8, CK18, etc. Molecular Markers • ER, PR, BCL2, MUC1, GATA-3, etc.	High molecular weight cytokeratins • CK5/6, CK14, CK17, CK19, etc. Molecular Markers • EGFR, C-KIT, CAVEOLIN-1, S-100, SMA, p63, p-CADHERIN, etc.

Referring back to the 5 subgroups of breast cancer defined by gene profiling, in the Luminal cancer subgroup, the tumor cells have immunophenotypic features shared with the normal luminal epithelial cells like expression of ER, PR, GATA-3 and the luminal cytokeratins CK8 and CK18. The Basal-like subgroup of cancers describes tumor composed of cells with variable expression of high molecular weight cytokeratins (CK5/6, CK14, CK17) and other molecular markers associated with the basal-like cell including EGFR, c-kit, caveolin1, etc... [7] For the HER2 subgroups, tumor cells have over-expression of HER2 and its related genes. Normal Breast-like breast cancer tends to express genes of the basal-like group and have low expression of luminal cell genes.

MOLECULAR SUBTYPES AND RESPONSE PATTERNS

The molecular subtypes may predict patterns of response to particular therapies. For example, luminal types, particularly Luminal A cancers, should be sensitive to endocrine therapy like tamoxifen and aromatase inhibitors. The HER2 positive group should be sensitive to monoclonal antibodies (trastuzumab) or small molecule tyrosine kinase inhibitors of Her2 like lapitinib.

Basal-like breast cancers with ER, PR and HER2 negativity were not previously recognized to have a therapeutic target but gene profiling and immunohistochemistry have revealed potential targets for (commercially) available drugs. Such targets include EGFR, c-kit, caveolin1, VEGF and poly ADP ribose polymerase (PARP). [14]

Already developed and marketed drugs can target these marker molecules and are being actively studied in TN cancers and pharmaceutical pipelines have focused much research and development to address drug discovery in this area.

RELATIONSHIP OF BASAL-LIKE BREAST CANCER AND THE TRIPLE NEGATIVE PHENOTYPE

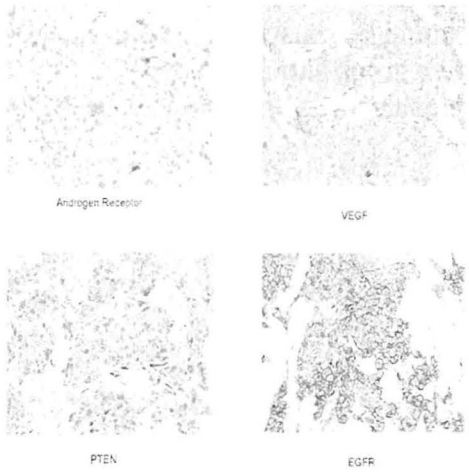
The term triple negative refers to ER, PR and HER2 negative tumors as defined by immunohistochemical staining. The term basal-like describes a molecular genotype based on gene profiling with expression of basal cytokeratins and other molecular markers. Most TN breast cancers tightly cluster within the basal-like subgroup on microarray. The terms basal-like and TN may be used interchangeably but they are not synonymous. There are significant differences between the two groups including cytokeratin (CK5/6, CK14, CK17) and other molecular marker expression like EGFR, c-kit, caveolin1, etc... [15]

In general, most basal-like breast cancer will be triple negative and most triple negative cancer will cluster in the basal-like group on MA. Currently, no clear cut, universally accepted definition for either of these terms exists. So, basal-like breast cancer contains a spectrum of tumors with TN as one of the subtypes.

THE CLINIC -- PRACTICAL APPLICATIONS

Gene expression profiles (MA) are not readily available for clinical use due to cost, technical difficulty and the need for fresh or frozen tumor tissue for the assay. Formalin fixed paraffin embedded tissue cannot be used. However, immunohistochemical (IHC) assays are automated, less costly, use formalin fixed tissue and are adapted for routine use in the clinical setting. These assays are used to categorize breast cancer specimens according to ER, PR, HER2, and CK expression (CK8, CK18, CK5/6, CK 14, CK17, etc…). Hence, IHC results have become the surrogate for selected gene expression for TN and basal-like breast cancers.

TUMOR IMMUNOHISTOCHEMISTRY TRIPLE NEGATIVE PROFILE



Basal Breast Cancer Profile

Biomarker	IHC Expression	% Positive Average	Staining Intensity
Androgen Receptor	Negative	0 %	1
VEGF	Positive	100 %	2+
PTEN	Positive	100 %	2+
EGFR	Positive	94 %	2.9

CLINICAL IMPORTANCE OF TRIPLE NEGATIVE BREAST CANCER CORRELATIVE CLINICAL CHARACTERISTICS

As a group TN tumors are aggressive with rapid growth rates and clinically tumors often present in between annual screening mammograms and are termed "interval tumors". [16]. Triple negative and basal-like breast cancers affect women of younger age, often premenopausal, especially compared to other breast cancer subtypes and are over represented in women younger than 30 years of age at breast cancer diagnosis. The TN phenotype can be found in variable percentages in all nationalities but has a higher incidence rate in women of African and African-American heritage. [17, 18] These features are strikingly displayed in a study of 148 Nigerian women with breast cancer. Two thirds of the patients were premenopausal with a mean age at diagnosis of 43.8 years and 59% had basal-like breast cancer and only 22% were ER positive. [19]

These TN tumors typically are high grade and have greater mean tumor size. There are conflicting reports as to whether they have an increased incidence of nodal metastatic involvement. [20]

Clinical series report higher rates of local, regional, and distant relapse particularly in the first 5 years after diagnosis with a peak incidence at year 3. The pattern of distant metastatic spread has a predilection for visceral and cerebral metastases. [21] Triple negative status has been reported as the greatest risk factor to develop brain metastases even exceeding that of HER2 positive breast cancers. The median time interval from original diagnosis to brain metastases was 22 months in one series and despite treatment, median survival was short at 4 months. [22]

Triple negative tumors also carry a higher risk for visceral spread compared to bony metastases. All these features link triple negative breast cancer to shorter disease free and overall survival compared to other breast cancer subtypes.

Triple negative tumors display a spectrum of response to chemotherapy with some tumors never relapsing after adjuvant or neo-adjuvant therapy but also a group that has higher rates of relapse and death within the first 3 to 5 years after diagnosis. [23] After relapsing, TN tumors respond temporarily to further treatment but rapidly develop resistance to further therapy and the patient dies of progressive metastatic disease with the TN group having a disproportionate death rate compared to other breast cancer types. [24, 25].

CLINICAL IMPORTANCE OF TRIPLE NEGATIVE BREAST CANCER PATHOLOGIC CHARACTERISTICS

Triple negative tumors have high histologic and nuclear grade compared to other tumor types and higher proliferative rates (elevated Ki-67 or MIB-1) and are ER, PR and HER2 negative. The tumors have high mitotic rates and many have abnormal p53 expression. Case series report EGFR expression in up to 60% of TN and BLBC and another 20 to 30% will express c-kit or caveolin1.Due to the overlapping relationship between basal-like breast cancer and TN phenotypes, immunohistochemical staining for cytokeratins in the TN tumors will have variable expression of the basal cytokeratins (CK5/6, CK14, CK17) as does the expression of other molecular markers like EGFR, c-kit, caveolin1, p63, etc… [2, 12, 26]

There are more unusual histologic types that are TN including metaplastic, medullary both typical and atypical, adenoid cystic and sarcomatoid breast cancers.

TN BREAST CANCER FEATURES	
Clinical	Pathologic
• Interval tumor (between mammograms) • Aggressive • Younger age onset • Premenopausal • Visceral metastasis • Cerebral metastasis • Local relapse risk • African and Afro American women predilection • Poorer prognosis for disease free and overall survival • Primary tumor type in BRCA1 mutation carriers	• High histologic/nuclear grade • CK 5/6, 14, 17 • High proliferative rate • Central necrosis/pushing border • ER, PR, HER2 negative • Ductal, metaplastic, medullary histology • Abnormal p53 • Expression EGFR, c-kit, caveolin1, p63, etc…

EPIDEMIOLOGY AND RISK FACTORS

There is a growing body of data regarding the epidemiology of TN breast cancer. From US and world-wide reports based on tumor registry data and population based case controlled studies, the two factors most consistently associated with the incidence of TN disease are age and race. TN cancers are not evenly distributed across races but occur predominantly in black women of any background as compared to the non-black woman.[27] This racial distribution has been confirmed in multiple reports. [17, 18, 28-30]

Thus, it has been observed that for premenopausal Afro-American women, basal-like or TN breast cancer represents 27 to 47% of their breast cancer subtypes while postmenopausal non- Afro-American women have only 14% basal-like or TN cancers. Overall black women have 2 to 3 fold more TN tumors than non-black women.

Additionally, age has emerged as an important factor in most studies with young age at menarche and younger age at first live birth associated with a higher incidence of TN disease. [30] And, lower economic status has been linked with incidence.

Multiple risk factors for the development of TN breast cancer have been described from many reports. [2, 15, 20, 31] The risk factors may vary depending on the population studied but are relatively consistent.

RISK FACTORS

- younger age at menarche
- younger age at first pregnancy
- higher parity
- shorter duration of breast feeding
- obesity defined by high BMI or WHR (waist–hip ratio)
- African or African-American race
- BRCA1 mutation carrier

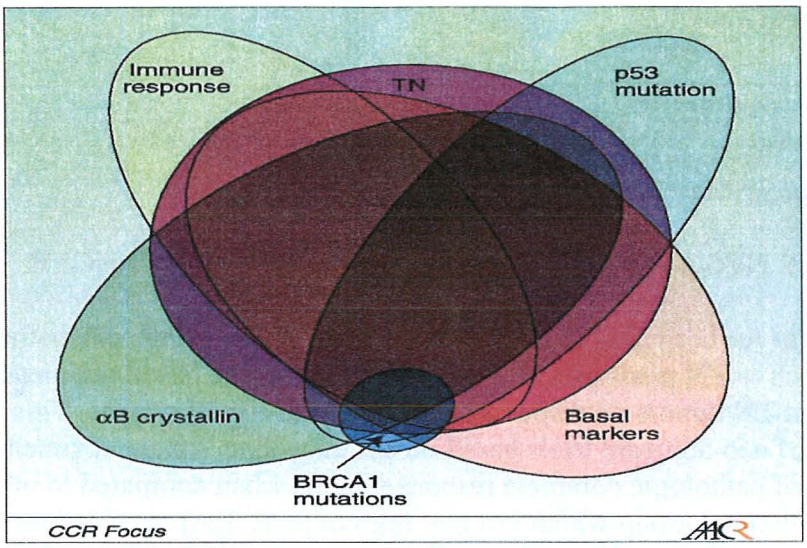
Modification of these risk factors to lower risk might include delayed onset of first pregnancy, encouragement of longer periods of breast feeding and decreasing abdominal obesity by diet modification and exercise.

A final risk for development of TN breast cancer is found in the BRCA1 mutation carrier since the TN phenotype and basal-like cancers are found in about 80% of the breast tumors arising in this high risk group.

TRIPLE NEGATIVE, BASAL-LIKE BREAST CANCER AND BRCA1 MUTATIONS

Carriers of germ-line BRCA1 mutations are known to be at high risk to develop breast cancer. These BRCA1 related tumors display a TN phenotype in a high percentage of cases and are predominantly basal-like in cytokeratin expression (CK5/6, CK14, CK17) and other basal markers (EGFR, P-cadherin, c-kit, etc…). [32-34] On gene arrays, BRCA1 breast cancers cluster

in the basal-like subgroup. [10] So, BRCA1 breast cancers and "sporadic" TN and basal-like breast cancers have similar immunophenotypic and gene expression. Despite this similarity, most TN and basal-like tumors do not have a mutation in the BRCA1 gene and are termed "sporadic" and while not mutated, the BRCA1 gene or its protein function may be dysfunctional or have low expression. [35-37]

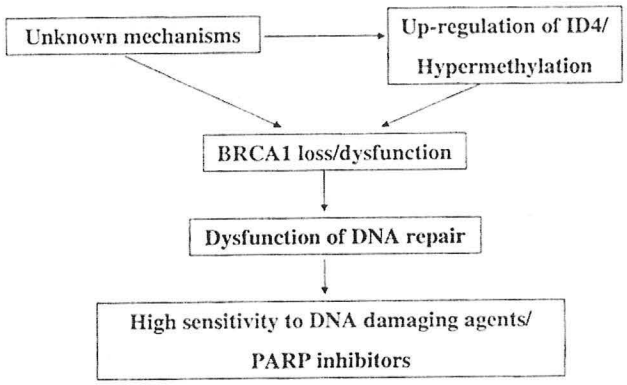


Schneider, B. P. et al. Clin Cancer Res 2008;14:8010-8018

BRCA1 GENE FUNCTION

The BRCA gene is involved in a variety of cellular functions including DNA double strand break repair by homologous recombination. Based on gene expression similarities between BRCA1 breast cancers and sporadic TN and basal-like breast cancers, it is postulated that BRCA1 dysfunction may be a shared characteristic of the sporadic TN and basal-like cancers and involved in their pathogenesis. [2, 3] Therefore, loss of BRCA1 function may play a significant role in the development of TN and basal-like breast cancer.

BRCA1 dysfunction may be caused by a variety of mechanisms. Promoter methylation may cause BRCA1 down regulation but this has not been identified in the majority of sporadic TN and basal-like cancers except for metaplastic or medullary tumors. [38, 39] Another potential mechanism is a 9 fold increased expression of ID4, a gene whose proteins are negative regulators (inhibitors) of BRCA1 function as reported in one study. [40] Yet, the predominant pathways of BRCA1 dysfunction are not known for the majority of sporadic TN and BLBC. Nonetheless, understanding and exploiting BRCA1 pathway dysfunction may yield treatment options. And, this year results of 2 different clinical trials in TNBC and BRCA1 cancers targeting the impaired BRCA1 pathway were announced with positive and exciting results.



Kurebayashi, J. Breast Cancer May 2009

TREATMENT OF TRIPLE NEGATIVE AND BASAL-LIKE BREAST CANCER

With lack of a receptor for hormonal or HER2 targeted agents, cytotoxic chemotherapy has been the primary approach to TN treatment. Anthracycline and taxane based regimens have been a standard approach and TN tumors do respond but have a high risk of relapse. This risk is clearly shown in the results of neo-adjuvant trials based on anthracycline- taxane regimens as TN tumors may have high rates of pathologic complete response (pCR) when compared to other tumor types particularly the luminal group which has low rates of pCR. [24] Achievement of a pCR is associated with long term disease free and overall survival. But, TN patients who do not have a pCR after neo-adjuvant therapy are more likely to relapse usually within the first 3 to 5 years post treatment. [23] Then they have a worse prognosis compared to the other tumor types. [25] Once relapsed, TN tumors tend to be aggressive even at first metastatic presentation. Involvement of difficult to treat visceral sites like lung, pleura and liver is common as is rapid development of brain metastases. [23, 24, 41] Responses to standard systemic therapy either single agent or drug combinations are limited with short term benefit before emergence of tumor resistance and progression. [42]

In an attempt to improve such dismal outcome, current treatment approaches are investigating different types of chemotherapy agents and the incorporation of biological therapies.

RETHINKING CYTOTOXIC DRUG CHOICES

The relation between BLBC and TN and BRCA1 tumors and the shared characteristic of BRCA1 dysfunction has stimulated investigators into new treatment options exploiting the dysfunctional BRCA1 pathway. Recent attention has focused on selecting chemotherapeutic agents with the potential to inflict a drug injury that a BRCA1 mutated or dysfunctional cell cannot repair. Platinum salts (cis and carboplatin) damage DNA by causing inter and intra-strand cross links that break the DNA strand and halt transcription. Triple negative tumors with a BRCA1 pathway of DNA repair that is either mutated or dysfunctional have increased sensitivity to DNA damaging agents like the platinums. [43-45] Multiple trials are underway to evaluate the efficacy of these agents in both TN and BRCA1 mutation cancers. A Phase II neo-adjuvant study in sporadic TN cancers treated with single agent cis-platin reported a 22% pCR rate. [46]

A second neo-adjuvant study of 25 TN patients with BRCA mutations had a 72% pCR to single agent cis-platin. [47] These results validate the concept of selective DNA damaging agent effectiveness with an impaired BRCA pathway.

In addition to platinum salts, other agents with DNA damage potential include etoposide, mitomycin C, irinotecan and temazolamide. These drugs are not normally included in the breast cancer armamentarium but are now being studied in clinical trials.

TRIPLE NEGATIVE BREAST CANCER TREATMENT
TARGETED THERAPIES

A variety of molecular markers are expressed in BLBC and TN tumors and identified by gene array. Clinical trials of targeted therapies using investigational agents are in progress. Some of the therapeutic strategies for TN and BLBC are:

1. Anti-angiogenic therapy – The vascular endothelial growth factor (VEGF) has been shown to be an important molecular target in breast cancers. A randomized Phase III trial of bevacizumab (anti-VEGF monoclonal antibody) plus weekly paclitaxel versus paclitaxel alone in metastatic breast cancer showed an improved response rate and progression free survival for the combination over the single agent. On subset analysis, the hormone receptor negative group (TN) treated with the combination experienced significant benefit in PFS (hazard ratio 0.5). [48] These results led to FDA approval and widespread use of bevacizumab for first line metastatic therapy in TN cancers. A large cooperative group study (ECOG 5103) is now investigating the addition of bevacizumab in the adjuvant setting with a planned analysis of the TN group response.[49] Several other agents with anti-angiogenic activity (sunitinib, sorafenib, pazopanib, etc...) are being evaluated in trials and may be more active due to their ability to hit multiple tumor targets.

2. Epidermal Growth Factor Receptor (EGFR) – EGFR is expressed in up to 60% of TN and BLBC. [50] EGFR activating gene mutations are rare and EGFR gene amplification occurs in about 25% of TN metaplastic breast cancers. Cetuximab, a monoclonal antibody against EGFR, has trivial response rate as a single agent in TN cancers but combined with carboplatin a 17% response rate was reported with an additional 31% clinical benefit rate. [51] A trial of carboplatin and irinotecan with cetuximab reported a 49% response rate in the TN tumors.[52] While these results are provocative, the role of EGFR targeting is yet to be defined for TN or BLBC and more study is planned.

3. SRC-ABL Inhibitors – Src tyrosine kinases are involved in a variety of cellular processes including proliferation, differentiation, and survival. Src is over-expressed in both TN and BLBC and is a potential target for therapy. [53] Dasatinib, an oral multi-targeted kinase inhibitor of Src family kinases and BCR-ABL, has in vivo and in vitro activity against TN tumors. Dasatinib has FDA approval for treatment of refractory CML and Philadelphia chromosome positive ALL. A recent study reported the Phase 1 data of oral dasatininb combined with capecitabine based on preclinical models suggesting drug synergy for TN tumors and Phase ll trials are planned. [54]

4. Caveolin1 – Caveolin1 is a main component of caveolar membranes functioning as a transport mechanism for molecule and drug access into cells. Caveolin1 is preferentially expressed in TN and BLBC and associated with an aggressive clinical course and poor outcome. [55, 56] ABI-007 (abraxane) is an albumin bound paclitaxel that selectively binds to caveolin and has enhanced drug delivery into the cell resulting in increased intracellular drug concentration compared to unbound paclitaxel. A Phase III trial of abraxane versus unbound paclitaxel showed a higher response rate (33% vs 19%) suggesting a selective therapeutic role for this drug in TN disease.[57] Phase II trials of ABI-007 plus bevacizumab and carboplatin are ongoing for TNBC.[49]

5. Poly (ADP-ribose) Polymerase (PARP) Inhibitors -- – The Synthetic Lethal Approach on a daily basis, environmental factors cause cellular DNA damage. This damage includes base changes, single and double strand breaks and inter and intra-strand cross linkage and is repaired by a variety of mechanisms including the BRCA double strand repair pathway. Single strand breaks are repaired by a base excision repair (BER) pathway of which PARP1 is an enzymatic component. PARP1 binds to DNA breaks and recruits other repair proteins. Inhibition of PARP1 causes loss of single strand repair but does not affect double strand repair. If the cancer cell has defective double strand repair (via BRCA1 pathway) and then single strand repair is blocked (PARP inhibition) then the effect is lethal for the cell. This double effect is termed Synthetic Lethality when the mutation in either of 2 individual genes has no effect but combining the mutations leads to cell death. [58] This rationale predicts that PARP inhibitors will be extremely effective in BRCA1 mutation cancers. For sporadic TN and BLBC, those tumors with impaired BRCA1 function should be susceptible to PARP inhibition.[59]

Gene *X*	Gene *Y*	
+	+	No effect
—	+	No effect
+	—	No effect
—	—	Death

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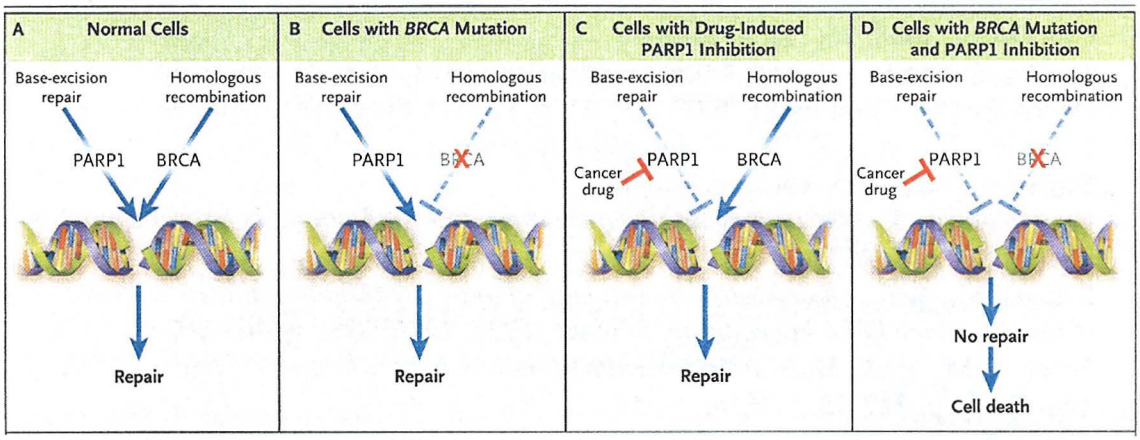
Recently announced clinical data from 2 different PARP inhibitor trials was presented. In the first study, 116 women with metastatic TN breast cancer received gemcitabine and carboplatin with or without BSI-201 an intravenous PARP1 inhibitor. Adding BSI-210 prolonged the median survival (9.2 vs 5.7 months) and increased the response rates (48% vs 16%) and gave 62% of women clinical benefit lasting at least 6 months vs 16% of the women on the chemotherapy alone arm. [60] Using a different PARP inhibitor, a trial of 54 heavily pretreated BRCA mutation patients with advanced breast cancer were treated with olaparib an oral PARP inhibitor. At high doses, olaparib had a 41% complete response rate and a 37% partial response rate with median time to progression of 5.7 months. [61] Hence, these trials confirm proof of principle for synthetic lethality in the clinical arena and offer an exciting treatment approach. However, much work still needs to be done and Phase III trials are planned.

Mechanism of Cell Death from Synthetic Lethality as induced by inhibition of Poly (Adenosine Diphosphate Ribose) Polymerase1 (PARP1)



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CONCLUSION

Breast cancer is not a single entity but a stunning array of molecular heterogeneity. Despite heterogeneity, there are reproducibly defined molecular subgroups that share gene expression. Within these subgroups, molecular targets have been defined for therapeutic treatment approaches. The concept that "one drug fits all" is clearly passé. Future clinical trials will consist of enriched patient populations for the molecular target being studied. The selection of chemotherapy agents, if used at all, will be based on an understanding of growth pathway survival and vulnerability for the different subgroups. And, for particularly aggressive malignancies like that of the TN and BLBC, there will be increased optimism that we can improve the prognosis for this group of women.

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