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The annals of the recalcitrant triple negative breast cancer: Classifications and its potential ameliorative measures

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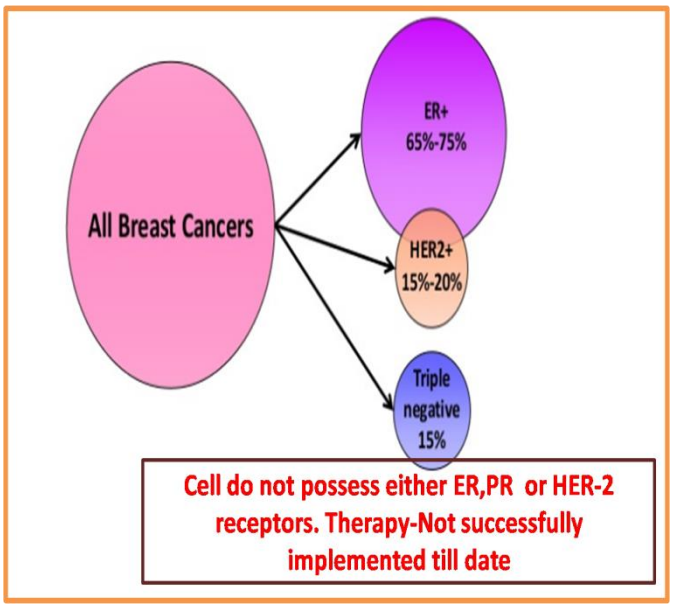
Graphical Abstract



Abstract.

Breast cancer is a heterogeneous disease that can be classified into different clinical, histopathological and molecular subtypes. 'Triple-negative' breast cancer (TNBC) comprises about 15% of all breast cancer cases and are devoid of the estrogen receptor (ER), progesterone receptor (PR) and the human epidermal growth factor receptor 2 (HER2) is over-expressed. Triple Negative Breast cancer is differentiated by **Brenton *et al* 2005** on the basis of prognosis and response to the therapy. The treatment that was possible till date for TNBC was chemotherapy as these can breast cancer cells typically lack ER, PR and express ex HER2 Hence the patients are unable to receive any conventional endocrine therapies .It is quite intriguing for for scientific and translational researcher, as TN phenotype

initially appears as a potential surrogate for basal-like breast cancers. ER and HER2 mRNA expression are found in basal/ myoepithelial cells of the normal breast, but one of the 'intrinsic gene' subtypes of the disease TNBC is characterized by the lack of these ER and HER2 mRNA expression. According, to **Perou *et al* 2000** TNBC are lacking ER and PR expression and HER2 over-expression /*HER2* gene amplification.

TNBCs usually exhibit quite high belligerent behaviour and is predominantly found in young women of Hispanic and African descent and a considerable link with *BRCA1* germline mutations has also been detected. These TNBCs exhibit quite high metastasis which culminates in the death of the patient within 5 years after diagnosis. However, TNBC is vastly heterogeneous and best considered as an umbrella term which comprises of different entities with characteristic genetic, histological, transcriptional, and clinical attributes. Till date, no effective treatment for metastatic TNBC is available following surgery, radiation and chemotherapy

Keywords Triple negative breast cancer, classifications, potential therapy.

Introduction

According to the survey of 2018, breast cancer death increased upto 15%. It has etiologies, with two type clinical histopathological and molecular subtypes. 'Triple-negative' breast cancer (TNBC) accounts for ~15% of all breast cancer cases. Most of the reason responsible for these tumors are over expression of epidermal growth factor receptor 2 (HER2) and inhibiting the expression of express estrogen receptor (ER), progesterone receptor (PR). TNBC have a more aggressive clinical course than other forms of breast cancer. TNBC has poor prognosis with highly complex genomic landscape. It has high risk to metastasis and death may occur within 5 years even after diagnosis.

Triple Negative Breast cancer is sub classified by **Brenton et al. (2005)** in the basis of prognosis and response to the therapy. Only treatment for TNBC was chemotherapy as they lack ER, PR and for excess HER2 patients are unable to receive endocrine and anti-HER2 therapies. It is inquisitive for scientific and translational researcher, as TN phenotype initially apparent as a potential surrogate for basal-like breast cancers. ER and HER2 mRNA expression are found in basal/myoepithelial cells of the normal breast, but one of the 'intrinsic gene' subtypes of the disease TNBC characterized by lack of these ER and HER2 mRNA expression. According, to **Perou et al. (2000)** TNBC are lacking ER and PR expression and HER2 over-expression /*HER2* gene amplification.

TNBCs arise from *BRCA1 and BRCA2* germline mutation. Mutation at 19p13.1 and MDM4 loci made TNBC idiosyncratic from other BC.TNBC with its heterogeneous character can be segregated into high and low grade.

GENETIC HETEROGENEITY OF TNBC

Given that the unifying feature of TNBCs is the lack of three biomarkers, the TNBC cells are quite heterogeneous in nature. **Kandoth, C. *et al. (2013)*** demonstrated that TNBCs have attributes like enormously high levels of genetic instability, with a median of 1.7 (range 0.16–5.23) mutations/Mb, and innumerable patterns of deviations in the copy number and structural rearrangements. Unlike other forms of breast cancer, where different genes have been found to be mutated in less than 10% of cases, the somatic gene mutations are found in more than 10% cases of TNBC predominantly in the genes like *TP53* and *PIK3CA* which is quite similar to that of the ER-positive breast cancers. Additionally these, TNBCs exhibit a considerable diversity in the mutational patterns. In many cases of TNBCs, an enhanced mutational rate highly modulates the different related cell signaling pathways. Unlike the ER-positive carcinomas, in TNBC the mutations in the *TP53* somatic genes mostly occur in the nonsense single-nucleotide variants. **Shah et al. (2012)** exhibited that the somatic mutations also affects other important cancer regulatory genes like *PTEN, RB1, NF1, BRCA1, BRCA2, ERBB3, ERBB4, ALK*, in some small groups of TNBC cells.

The heterogeneity in mutations of TNBCs is closely associated with variations in their clonal composition with different clones present in a single tumor and multiple clones in some cases. The mutations in the *TP53*, *PIK3CA*, and *PTEN* genes are present in the early stages of tumor development. On the other hand the mutations that regulates cellular migrations and epithelial-to-mesenchymal transition exhibit much diminished rate of frequencies, clearly indicating that they occur in the later stages of tumor development.

TNBCs, as a group, display complex patterns of CNAs, with multiples gains and losses intercalated across all the chromosomes and few focal high-level amplification. Recurrent CNAs found in TNBCs include gains of 1q, 8q, and 10p, and losses of 5q and 8p, as well as *PARK2* intragenic deletions, *EGFR* and *FGFR2* amplifications, and *PTEN* loss reported by **Turner et al. (2010).** Notably, TNBCs lack concurrent 1q gains and 16q losses, changes typically found in ER-positive breast cancers.

The recurrence of the functional gene rearrangements have been well documented in TNBC.A major subset of TNBCs encompassing rearrangements of the Notch genes (*NOTCH1* and *NOTCH2*) and microtubule-associated serine-threonine kinase genes (*MAST1* and *MAST2*). These findings may open new vistas for therapeutic strategies avenues. This has been already depicted in patient-derived xenograft models of Notch-altered tumors and cell lines bearing fused Notch genes. The results suggest that the progression of TNBC is sensitive to the inhibition of the Notch signaling pathway. Furthermore, **Banerji et al. (2012)** reported the occurrence of *MAGI3–AKT3* fusion gene in TNBC, which was observed in 7% of TNBC cases (5/72) with rhe aid of reverse transcription PCR (RT-PCR. **Banerji et al. (2012*)*** also reported the presence of the *MAGI3–AKT3* fusion gene in case of TNBC gene using a hybrid capture array.

Transcriptomic heterogeneity of TNBC

Although initially perceived similar to the basal-like breast cancer TNBCs exhibit considerable heterogeneity and that it is quite different from the basal like breast cancers. **Bastien et al. (2012)** proposed an intrinsic subtype of triple negative phenotype was denoted as claudin-low, which is characterised by attributes like low levels of luminal differentiation markers, is enriched for the expression of epithelial-to-mesenchymal transition, immune response and cancer stem cell-related genes.

The seminal studies carried out by **Lehmann *et al*.(2011)** further demonstrated the transcriptional heterogeneity of TNBCs, revealing the existence of **six subtypes** of TN disease: basal-like 1, basal-like 2, immunomodulatory, mesenchymal, mesenchymal stem-like and luminal androgen receptor (LAR).

Basal -like 1 subtype - Among the basal-like subtypes, the basal-like 1 subset was found to be enriched in cell division and DNA damage response pathways.

Basal –like 2 subtype- The basal-like 2 group displayed an association with growth factor signaling and myoepithelial marker expression.

Immunodulatory subtype- The immunomodulatory subtype is characterized by immune cell processes and immune signaling cascades.

Mesenchymal subtype - Although the mesenchymal stem-like and mesenchymal subtypes share several transcriptomic similarities and are enriched for genes implicated in cell motility and epithelial-to-mesenchymal transition.

Mesenchymal stem like subtype- The mesenchymal stem-like subtype displays lower expression of genes associated with cellular proliferation, and is enriched for genes related to mesenchymal stem cells.

Luminal androgen receptor- The LAR subtype displays a luminal-like gene expression pattern despite ER-negativity, most likely due to androgen receptor activation.

Comparative analyses of these six subtypes with the intrinsic gene subtypes revealed that basal-like 1, basal-like 2, immunomodulatory and mesenchymal TNBCs are preferentially of basal-like intrinsic subtype, that a large proportion of mesenchymal stem-like TNBCs fit the intrinsic normal-like or claudin-low subtypes, and that the LAR subgroup corresponds in most part to the rare TNBCs classified by PAM50 as luminal or HER2-enriched.

It should be noted that this six TNBC subtype classification may have therapeutic implications, given that -

(i) xenografts of breast cancer cell lines classified as of basal-like subtypes were found to be sensitive to platinum salts, whereas mesenchymal and LAR subtype xenografts were sensitive to PI3K/mTOR pathway inhibition and anti-androgen therapy, respectively; and

(ii) that approximately 50% of patients with basal-like 1 TNBCs were reported to evolve to pCR following standard neoadjuvant chemotherapy, whereas the pCR rates for other subgroups, such as LAR (10%) and basal-like 2 (0%), were found to be markedly lower.

Curtis et al. (2012) proposed an alternative taxonomy for breast cancer based on the integration of CNAs and gene expression profiles, which defined 10 integrative clusters (IntClust 1–10). IntClust 10 is composed mainly by poorly differentiated TNBCs, with highly recurrent *TP53* mutations and intermediate genomic instability, and is characterized by poor prognosis in the first 5 years after diagnosis. On the other hand, approximately a fourth of all TNBCs correspond to the IntClust 4 subtype, which has low levels of genomic instability, absence of CNAs, marked lymphocytic infiltrate and a better outcome, providing another level of evidence to demonstrate the genomic heterogeneity of TNBCs.

More recently, **Burstein et al. (2015)** put forward yet another gene expression classification, which categorizes TNBCs in luminal/androgen receptor **(LAR),** mesenchymal **(MES),** basal-like/immune-suppressed **(BLIS),** and basal-like/immune activated **(BLIA)** subtypes which have distinct clinical outcomes. The BLIS subset displays the best outcome, whereas the BLIA subgroup confers the poorest prognosis. Moreover, subtype-specific gene amplifications were detected. LAR, MES, BLIS, BLIA subtypes harbor amplifications of *CCND1*, *EGFR*, *FGFR2*, and *CDK1*, respectively.

Although 'Burstein's' and 'Lehmann's' LAR and mesenchymal subtypes showed significant overlap, Burstein's BLIS and BLIA subtypes were a mixture of Lehmann's groups), suggesting that not all TNBC gene expression subtypes are stable and reproducibly identified, as previously demonstrated for the intrinsic gene subtype classification.

THE HISTOLOGIC SUBTYPES

The large majority of TNBCs are high-grade invasive carcinomas of no special type displaying pushing invasive borders, central necrosis, brisk lymphocytic infiltrates, marked nuclear pleomorphism, and numerous mitoses. Nevertheless, there is a multitude of rare histologic special types of breast cancer that are consistently of TN phenotype.

Some high-grade special histologic types of breast cancer, including carcinomas with apocrine features, carcinomas with medullary features, and metaplastic breast carcinomas (MBCs) almost invariably display a TN phenotype. Notably, among TNBCs, carcinomas with apocrine features are the ones most likely to express androgen receptor and display a molecular apocrine or LAR gene expression profile reported by **Montagna et al. (2013).** Thus, their identification may suggest potential sensitivity to anti-androgen receptor agents and may trigger androgen receptor testing, but does not carry definite prognostic information as their outcome is uncertain and has been reported to be comparable to that of conventional invasive carcinomas of no special type. Likewise, contradictory data have been published

regarding the prognostic impact of androgen receptor expression in TNBCs. Medullary carcinoma is a controversial histologic special type of breast cancer, which has been reclassified as a histologic pattern (i.e., carcinomas with medullary features) in the latest World Health Organization classification. Well-circumscribed borders, a syncytial growth pattern, and brisk lymphocytic infiltrate are the hallmark features of the so-called medullary carcinoma; their histologic identification, however, has been shown to lack in inter-observer reproducibility. Despite worrisome cytological features and high mitotic activity, carcinomas with medullary features are historically perceived to have an excellent outcome reported by **Huober et al. (2012).** Given the low inter-observer agreement rate for the identification of this histologic subtype, a diagnosis of carcinoma with medullary features does not carry any therapeutic implication and patients with these cancers should be treated following the same protocols for common forms of TNBC. In fact, one could argue that the good prognosis historically reported to medullary carcinomas is merely a reflection of the brisk lymphocytic infiltrate that these tumors display, which has now been validated by level I evidence as a prognostic marker for patients with TNBC treated with chemotherapy.

MBCs encompass a spectrum of tumors with squamous and/or mesenchymal differentiation, are mostly high-grade lesions, display worse outcome than conventional TNBCs, and show significant inter- and intra-tumor heterogeneity. These tumors are preferentially classified by **Weigelt et al. (2015)** as of claudin-low or basal-like intrinsic subtype, however, there is evidence that their histologic subtype, as well as the subtype present in the sample subjected to molecular analysis, have an impact on their genomic profile. The spindle cell MBCs are preferentially of claudin-low intrinsic subtype, whereas the squamous and chondroid MBCs are classified more frequently as basal-like. At the genetic level, MBCs are enriched for genetic alterations affecting Wnt and PI3K pathways, in particular in the form of *PIK3CA* mutations. The data on the repertoire of genetic alterations in MBCs is scarce, however, our group has demonstrated that histologically distinct morphological components within individual MBCs may display distinct patterns of CNAs, despite being clonally related.

Although as a group TNBCs display an aggressive clinical behavior, a subset of these cancers are characterized by low histologic grade and an indolent behavior. For example, even among MBCs, low-grade variants exist, such as the low-grade spindle and adenosquamous carcinomas, which display a less aggressive clinical course. Among low-grade TN neoplasms, at least two subsets can be distinguished by **Lakhani et al. (2012)**: (i) carcinomas with salivary gland-like morphology, which are underpinned by specific/pathognomonic genetic alterations and display low-to-intermediate levels of genetic instability; (ii) a subgroup of low-grade lesions, including lesions once considered to be benign hyperplastic proliferations [i.e. microglandular adenosis (MGA), atypical lesions i.e., atypical

microglandular adenosis (AMGA)] and invasive carcinomas (i.e., acinic cell carcinoma (ACC)), that, despite their low-grade morphology and good outcome, recapitulate the complex genomic landscape of usual TNBCs.

SALIVARY GLAND-LIKE TUMORS OF THE BREAST

This group of TNBCs recapitulate neoplasms primary of the salivary glands not only morphologically, but also genetically. In contrast to conventional TNBCs, these tumors lack recurrent *TP53* aberrations, display few copy number alterations and harbor specific/pathognomonic genetic alterations. This group includes the well-characterized adenoid cystic carcinoma (AdCC) and secretory carcinoma, underpinned by *MYB–NFIB* and *ETV6–NTRK3* fusion genes, respectively**. Persson M. et al. (2009)** presents additional lesions rarely occurring in the breast, yet comprehensively studied when arising in the salivary glands, can tentatively be included in this subgroup, such as the polymorphous low-grade adenocarcinoma and mucoepidermoid carcinoma, which are characterized by *PRKD1* hotspot mutations and *MAML2*rearrangements, respectively. denomyoepitheliomas of the breast, though heterogeneous, are frequently of TN phenotype and can be morphologically identical to epithelial–myoepithelial carcinomas of the salivary glands. Recent data suggest that their phenotypic similarities may be underpinned by a similar constellation of mutations (Reis-Filho et al., manuscript under review).

Adenoid cystic carcinoma: AdCCs, albeit originally described in the salivary glands, can also arise in other anatomical sites, including the lungs and breast. Breast AdCCs account for less than 0.1% of breast carcinomas, and typically show a good prognosis, in contrast to the poor long-term outcomes in head and neck AdCCs. AdCCs are composed of a dual population of luminal and myoepithelial/basal cells, growing in cribriform, tubular and/or solid patterns proposed by **Marchio et al. (2010).** The vast *majority* (>95%) is TN and at the transcriptomic level they pertain to the basal-like subtype. No data are available in regards to AdCC and the TNBC gene expression classification by **Lehmann et al. (2011).**

Regardless of its anatomic location, the hallmark genetic alteration of AdCC is a rearrangement of the *MYB* gene, most frequently in the form of *MYB–NFIB* fusion gene, resulting in the *t*(6;9)(q22–23; p23–24) translocation. The prevalence of such alteration ranges from 23 to 100% in breast AdCCs. Notably, breast AdCCs lacking the *MYB–NFIB* rearrangement have been shown to be morphologically similar to those harboring this fusion gene. Distinct rearrangements affecting a second MYB family gene (*MYBL1*) have been demonstrated in AdCCs of other sites, acting in remarkably similar ways, and theoretically also detectable in breast AdCCs.

In contrast to common forms of TNBCs, AdCCs display quiet genomes lacking high-level amplifications or homozygous deletions, as well as CNAs frequently present in usual TNBCs, such as 8q gain and 5q loss. Breast AdCCs rather show recurrent 17q21-q25.1 gains and 12q12-q14.1 losses. Interestingly, AdCCs occurring in the salivary gland also display 12q13 losses. Similar to AdCCs of the salivary glands, the mutation rate of breast AdCCs is low. They lack *TP53* and *PIK3CA*somatic mutations and preferentially harbor mutations affecting genes associated with chromatin remodeling, cell adhesion and signaling cascades. Breast AdCCs have also been shown to display recurrent mutations in *TLN2*, *MYB*, and *BRAF* and to harbor mutations in cancer genes reported to be mutated in salivary glands AdCCs, such as *SF3B1*, *FBXW7*, and *FGFR2*.

High-grade transformation has been described in breast and salivary gland AdCCs). Notably, high-grade TNBCs arising in low-grade AdCCs or high-grade basaloid AdCCs may also harbor the *MYB–NFIB* fusion gene. **Seethala et al. (2007)** suggested that in salivary glands p53 or PTEN inactivation, or yet *MYC* amplification may have a role in this phenomenon. Our group has recently reported on two breast AdCCs with high-grade transformation; our findings confirmed that progression occurred via the acquisition of additional genetic events and/or clonal selection; however, none of the genetic alterations reported in the progression of salivary gland AdCCs were found in breast AdCCs. In a distinct study of a single breast AdCC metastasizing to the kidney, *PIK3CA* and *PTEN* mutations were found in both the primary and metastatic tumors, but, the metastatic deposits showed increased *PTEN* promoter methylation and lower *PTEN* gene expression levels. It seems therefore unlikely that a single genetic event is responsible for the high-grade transformation observed in human AdCCs.

Secretory carcinoma: Secretory carcinoma is a rare entity, accounting for less than 0.15% of breast cancers. Although initially described in children and named ‘juvenile carcinoma’, it was later shown to occur at a median age of 53 years. This entity has an excellent clinical outcome, with protracted survival even in the presence of nodal involvement and metastatic disease. **Horowitz et al. (2012)** said that morphologically it displays tubular, solid and/or microcystic growth patterns with intra- and extra-cellular dense eosinophilic secretions. Although the vast majority of cases are low-grade TNBCs, cases of high-grade or with weak hormone receptor expression have been reported.

Over 90% of secretory carcinomas harbor the *t*(12;15)(p13;q25) translocation resulting in the *ETV6–NTRK3* fusion gene. **Tognon et al. (2002)** showed that although this translocation also underpins a variety of neoplasms of other sites (i.e., infantile fibrosarcoma, cellular congenital mesoblastic nephroma, acute myelogenous leukemia), in the context of breast carcinomas it is pathognomonic of secretory carcinoma. Importantly, the ETV6–NTRK3 fusion protein can be inhibited by crizotinib and

other small molecule inhibitors, potentially offering a therapeutic strategy for the rare cases of metastatic and chemoresistant breast secretory carcinomas.

The salivary gland counterpart of breast secretory carcinoma was first recognized due to the discovery that *ETV6–NTRK3* translocation underpins lesions morphologically similar to breast secretory carcinomas but previously classified as unusual variants of salivary gland ACCs. These lesions were then renamed mammary analog secretory carcinoma. A later study found that these tumors may harbor *ETV6* rearrangements with an unknown partner (*ETV6-X*), which theoretically may also occur in the breast counterpart.

Secretory carcinomas have simple genomes with few CNAs. Recurrent 8q, 1q, 16pq, and 12p gains, along with 22q losses have been identified. **Del Castillo et al. (2015)** have reported on a case of a lethal high-grade secretory carcinoma with fluorescence in situ hybridization-proven *ETV6* rearrangement, which despite harboring a simple genome, displayed more gains and losses of entire chromosomes and chromosomal arms than lower-grade tumors. Further studies of secretory carcinomas with high-grade transformation are warranted to define their prognosis and molecular underpinning.

LOW-GRADE TN BREAST NEOPLASIA FAMILY

It is currently perceived that breast cancer evolution can be stratified into two main pathways according to ER pathway activation. ER-positive breast neoplasms encompass a spectrum of pre-invasive (columnar cell lesions, atypical ductal hyperplasia, lobular neoplasia, and low-grade ductal carcinoma *in situ*) and invasive lesions (invasive tubular, lobular, and low-grade ductal carcinomas), and progression from low- to high-grade lesions may take place. Owing to their frequent coexistence and similar pattern of genetic alterations (e.g., *PIK3CA* mutations and deletions of 16q and gains of 1q, low-grade ER-positive non-obligate precursors and invasive carcinomas have been grouped together under the term ‘low-grade breast neoplasia family’ determined by **Lopez-Garcia et al. (2010).**

Akin to the low-grade ER-positive breast neoplasia family, a subset of related low-grade neoplastic entities can also be distinguished among the TN lesions of the breast. Indeed, MGA, AMGA, and ACC show overlapping morphology and immunophenotype (lack of ER, PR, and HER2 and *expression* of S100 protein), and are characterized by nearly indistinguishable genomic landscapes to those of common forms of TNBC. When not associated with high-grade TNBCs, these lesions have an indolent clinical behavior and limited metastatic potential despite the worrisome genomic landscape, and should be managed accordingly. Progression to high-grade TNBCs, however, is not uncommon. Notably, in both ACC and MGA/AMGA, the development of metaplastic TNBC has been reported by **Khalifeh et**

al. (2008). We can therefore hypothesize within the ER-negative branch of breast cancer evolution the existence of a 'low-grade TN breast neoplasia family' comprising MGA, AMGA, and ACC, which may give rise to *bona fide* high-grade TNBCs.

Microglandular adenosis

MGA is histologically characterized by a haphazard proliferation of small glands infiltrating adipose and collagenous tissue, without eliciting a desmoplastic reaction. Although surrounded by a basement membrane, MGA acini lack a myoepithelial cell layer, in a way akin to invasive carcinomas. Although some have regarded MGA as a benign hyperplastic lesion, **Tavassoli et al. (1983)** recognized that MGA encompasses a spectrum of lesions including pure MGA without atypia, atypical MGA (AMGA), and MGA associated with invasive carcinoma. Notably, MGA, AMGA, and associated high-grade TNBCs display a similar phenotype, including the expression of S100 protein, and pattern of genetic alterations. These findings are consistent with the notion that MGA/AMGA is in fact clonal neoplastic lesions and non-obligate precursors of TNBCs.

As a group, MGA/AMGA display complex copy number profiles with recurrent 5q losses and 8q gains. Massively parallel sequencing analysis has revealed that these lesions harbor highly recurrent (~80%) *TP53* mutations and a vast repertoire of mutated genes at low frequency, including *BRCA1*, PI3K pathway genes (*PTEN*, *PIK3CA*, and *INPP4B*) and genes encoding for receptor tyrosine kinases (*ERBB3*, *FGFR2*). Significant heterogeneity, however, is observed. Current data favor that the majority of pure MGAs differ from carcinoma-associated MGA/AMGA, given that pure MGAs lack *TP53* mutations and copy number alterations affecting genomic regions commonly altered in TNBCs. It is therefore possible that acquisition of *TP53* mutations is a driver of progression of MGAs and that the early genetic alterations responsible for the development of these lesions have yet to be unveiled.

Treatments and Research for Triple-Negative Breast Cancer

Common Treatments for TNBC

The treatment is based on the extent of cancer progression, the size of the main tumor and details of pathology tests such as the tumor grade which reflects the rapidity of cell division. In case of the disease being detected at early stage, the patients will have time to undergo the processes like surgery, chemotherapy and radiation therapy.

Surgery

Surgery can be one of two types. Many doctors think that because this type of cancer is aggressive, it's best to do a mastectomy to remove the entire breast. This tends to happen if:

- Have several tumors
- The cancer is in the skin
- A tumor in the nipple
- Already had cancer in that breast
- The tumor is large
- There are calcium deposits or other abnormal cells in the breast

Chemotherapy

Chemotherapy is the most effective systemic, or whole-body, treatment for triple-negative breast cancer. The reason is that chemotherapy works better than other treatments to kill cancer cells that divide quickly, which is very common in triple-negative disease. Chemotherapy also helps prevent breast cancer cells from spreading, or metastasizing, to other parts of the body.

Chemotherapy, a medicine that kills cancer cells. Patients can get it by a needle into a vein or in a pill. When it's caught early, this type of cancer may respond better to chemo than others do. Patients are most likely to take two chemotherapy drugs over 12 weeks or three drugs over 18 weeks. Chemotherapy may shrink the growth of large tumors and make the operation easier.

POST NEO-ADJUVANT CHEMOTHERAPY OF TNBC

Although neoadjuvant systemic therapy does not improve the overall survival of breast cancer patients compared with adjuvant chemotherapy, the achievement of pathologic complete response (pCR) following neoadjuvant chemotherapy is associated with an improved prognosis. TNBCs display the highest rates of pCR following neoadjuvant chemotherapy, with approximately 35–50% of TNBCs achieving pCR following anthracycline+taxane neoadjuvant chemotherapy regimens. Importantly, the patients with TNBC who achieve pCR have been shown to have an excellent long-term clinical outcome, with very few distant relapses; on the other hand, patients who have residual disease after neoadjuvant chemotherapy have a poor prognosis.

A subset of TNBCs has been suggested to harbor homologous recombination DNA repair defects similar to those found in tumors arising in *BRCA1* and *BRCA2* mutation carriers. Given that tumors

with homologous recombination DNA repair defects may show greater sensitivity to agents that cause DNA double-strand breaks and crosslinks, such as platinum salts and inhibitors of the Poly(ADP) Ribose Polymerase (PARP), it has been posited that, as a group, patients with TNBCs may benefit from platinum-based chemotherapy reported by **Farmer et al. (2005).** There is burgeoning evidence to demonstrate that a subset of TNBC patients may benefit from the addition of platinum-based chemotherapy to current chemotherapy regimens. **Von Minckwitz et al. (2014)** demonstrated that the addition of carboplatin to doxorubicin and paclitaxel in patients with TNBC results in significantly higher rates of pCR than the current anthracycline+taxane-based chemotherapy (pCR rates 60% (54–66%) vs. 46% (40–53%) in the CALGB 40603 trial and 53.2% (54.4–60.9%) vs. 36.9% (29.4–44.5%) in the GeparSixto trial. Although the concept of BRCAness is known for over a decade, biomarkers to define which TNBC patients are likely to benefit from this regimen have yet to be fully developed.

Given that TNBC patients with residual disease after neoadjuvant chemotherapy have a shorter overall survival than patients with non-TN breast cancers, the identification of targetable alterations in TN residual disease is of paramount importance. **Balko et al. (2014)** have recently analyzed a series of 74 residual TNBCs following neoadjuvant chemotherapy and showed that >90% of cases had alterations in at least one clinically targetable pathway (*PTEN*, *JAK2*, *CDK6*, *CCND1*, *CCND2*, *CCND3* and *IGF1R*). In addition, a higher frequency of potentially targetable alterations was detected in post-treatment TNBCs compared with primary basal-like breast cancers from TCGA. A frequent *MYC* and *MCL1* co-amplification in residual TNBCs following neoadjuvant chemotherapy was detected, with *MCL1* gains in 83% of *MYC*-amplified cases. Moreover, concurrent forced expression of *MYC* and *MCL1* in MCF10A cells enhanced cellular colony formation, whereas their silencing increased cellular sensitivity to doxorubicin.
The same group has also identified in TNBCs following neoadjuvant chemotherapy down regulation of *DUSP4*, a phosphatase that negatively regulates members of the MAP-kinase pathways. Reduced expression of *DUSP4* was found to be associated with a worse outcome in TNBC patients, and when detected in TNBCs after neoadjuvant therapy DUSP6 reduced expression was associated with treatment-refractory high Ki67 scores and shorter recurrence-free survival. In addition, MEK inhibition synergized with docetaxel in TNBC xenografts, **Balko et al. (2012)** suggested that this therapeutic combination might potentially benefit TNBC patients with residual disease after neoadjuvant chemotherapy.

Taken together, neoadjuvant therapy of patients with TNBC has revealed that a subset of these cancers is remarkably sensitive to conventional cytotoxic agents and that this effect is increased by the addition of platinum salts. Opportunities for translational research in this area include the developments of

biomarkers to predict pCR in patients with TNBC, the analysis of post-neoadjuvant chemotherapy residual disease, and whether this residual disease differs from (micro) metastatic disease in these patients.

Neo-adjuvant vs. adjuvant chemotherapy

Chemotherapy for breast cancer can be given either:

- before surgery, which is called neoadjuvant therapy, or
- after surgery, which is called adjuvant therapy.

So far, research has found both options to be just as effective at lowering the risk of cancer return and prolonging life.

Most chemotherapy for breast cancer is given as adjuvant therapy, after and in addition to surgery. Therapy usually begins about a month after surgery, once you have had a chance to heal.

There are two possible benefits of neoadjuvant treatment:

- It may shrink the cancer so that patients have less extensive surgery.
    - If the cancer is very big patients may need treatment to reduce it before it can be removed.
    - Or lumpectomy instead of mastectomy.
- Getting chemotherapy before surgery gives information about how the cancer responds to treatment. If one chemotherapy medicine doesn't shrink the cancer, doctor will have information to use when choosing which medicine to try next.

Radiation

Radiation is a local therapy that kills any cancer cells left in areas of the body after surgery at high risk for cancer return. Those areas may be in the breast or chest wall, with or without the nearby lymphnodes. Adjuvant radiation therapy helps protect patient from the breast cancer coming back in the treated areas, also called local recurrence.

Radiation is usually given after lumpectomy. If patient need a mastectomy, doctor may recommend radiation therapy to reduce the chance of the cancer coming back in the skin or the chest wall or nearby lymph nodes if:

- the primary tumor is larger than 5 centimeters across

- the cancer involves the lymph nodes under the arms
- the cancer has grown into the skin or chest wall muscle under the breast
- the triple-negative breast cancer is locally advanced or inflammatory breast cancer

Natural Treatment for TNBC

Hormonal receptors lacking render the conventional breast cancer drugs redundant, forcing scientists to identify novel targets for treatment of TNBC. Two natural compounds, curcumin and resveratrol, have been widely reported to have anticancer properties. *In vitro* and *in vivo* studies show promising results, though their effectiveness in clinical settings has been less than satisfactory, owing to their feeble pharmacokinetics. Here we discuss these naturally occurring compounds, their mechanism as anticancer agents, their shortcomings in translational research, and possible methodology to improve their pharmacokinetics /pharmacodynamics with advanced drug delivery systems.

Curcumin (diferuloymethane) is an extract of the rhizome of turmeric (*Curcuma longa* Linn) an Indian traditional medicine exhibiting antiangiogenic, antiproliferative, anti-tumorigenic, antioxidant, and anti-inflammatory properties in both *in vitro* and *in vivo* studies.

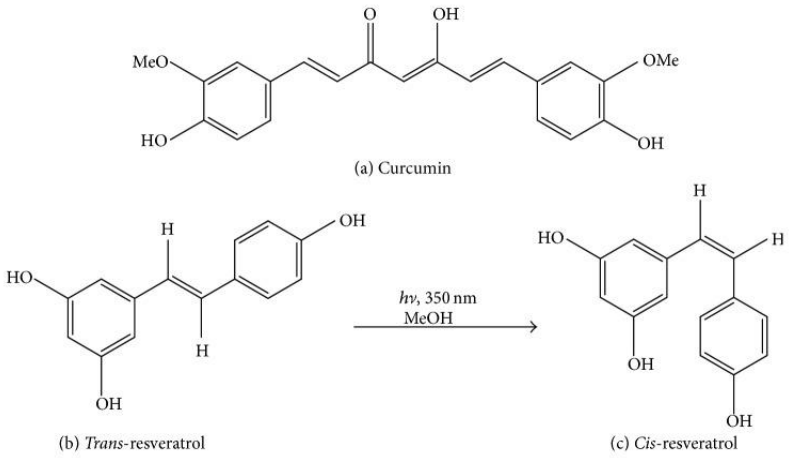


Figure 5: structure of (a) Curcumin (b) Trans-resveratrol (c) Cis-resveratrol

Resveratrol is a naturally occurring polyphenol that has been reported as a cardioprotective, neuroprotective, chemopreventive agent, along with antiageing properties. Studies have been carried out using *in vitro* and animal models for studying the action of resveratrol on cancer cells and cancer related pathways.

Advanced drug delivery system (ADDS)

To overcome the problems of poor bioavailability and poor pharmacokinetics associated with curcumin and resveratrol, numerous ADDS systems like adjuvants, nanoparticles, liposomes, micelles, phospholipid complexes, dendrimers, nanoemulsions, nanogels, and nanogold are being developed by performing extensive studies.

Curcumin

Adjuvants are known to have an inhibitory activity on hepatic and intestinal glucuronidation. Use of piperine as an adjuvant with curcumin was administered in rats and healthy human volunteers. 2 g/kg of curcumin alone showed a maximum serum curcumin level of 1.35 (0.23 μg/mL at 0.83 h), whereas concomitant administration of piperine (20 mg/kg) increased the serum concentration of curcumin for a short period of time with a significant increase in its maximum peak level.

Resveratrol

It has been known that "β-glucan" has been used as an adjuvant, drug carrier, or in combination with a drug or compound such as resveratrol by developing drug delivery system to enhance the bioavailability. A study was performed by a laboratory to know the possible combination/synergistic effects of β-glucan and resveratrol on immune reactions.

Nanotechnology (Nanoparticles)

Nanoparticle technology is a promising drug delivery system developed to enhance the bioavailability of many therapeutic drugs especially highly hydrophobic agents like curcumin.

Limited studies have shown the application of curcumin nanoparticles. Bisht et al. reported the synthesis, physicochemical properties, and cancer related application of "nanocurcumin" (size less than 100 nm), a polymer-based nanoparticle of curcumin. In a study it was reported that nanocurcumin showed similar *in vitro* activity as that of free curcumin in pancreatic cell lines. Solid lipid nanoparticles (SLNs) which are known as lipid-based drug delivery have become an area of focus in recent times.

The use of resveratrol in combination with serum albumin in a nanoformulation has been found to significantly inhibit the growth rate of human primary ovarian cancer cells as compared to free resveratrol when implanted subcutaneously. **Shao et al. (2009)** used mPEG poly(epsiloncaprolactone)-based nanoparticles incorporating resveratrol and demonstrated a significantly higher rate of cell death as compared to an equivalent dose of free resveratrol in glioma cells.

In a study, researchers used cyclodextrin-based nanosponges to enhance curcumin's solubility. They employed dimethyl carbonate as a cross-linker and formulated the complex of β-cyclodextrin-curcumin

nanosponge. The solubilization efficiency of loaded nanosponges was found to be more compared to free curcumin and *β*-cyclodextrin complex. Interactions of curcumin with nanosponges were confirmed by the characterization of curcumin nanosponge complex. Also, drug release of curcumin in *in vitro* studies was well controlled over a prolonged time period and the complex was found to be nonhemolytic reported by **Darandale et al. (2013).**

Liposomes

Liposomes carry both hydrophilic and hydrophobic molecules and are known as excellent drug delivery systems.

In vitro and *in vivo* antitumor activity against human pancreatic carcinoma cells using liposomal curcumin demonstrated that liposomal curcumin suppressed the pancreatic carcinoma growth in xenograft models by inhibiting tumor angiogenesis.

In another study, *in vitro* and *in vivo* effects of liposomal curcumin on proliferation, apoptosis, signaling, and angiogenesis in human pancreatic carcinoma cells were studied. **Li et al. (2005)** showed NFk-B downregulation, growth suppression, and apoptosis induction *in vitro*. *In vivo* results reported antitumor and antiangiogenesis activity as well.

In one study, when mitochondrial targeting resveratrol liposomes were used, this induced apoptosis in both nonresistant and resistant cancer cells by dissipating mitochondrial membrane potential. It also increased caspase-9 and caspase-3 activities. Significant antitumor efficacy was exerted by resveratrol liposomes in in xenografted resistant A549/cDDP cancers in nude mice and tumour spheroids by deep penetration.

Polymeric Micelles and Phospholipids Complexes

Micelles and phospholipid complexes can improve the gastrointestinal absorption of natural compounds by decreasing undesirable rapid metabolism and early elimination resulting in higher plasma levels and improved bioavailability.

In vitro model of everted rat intestinal sacs, intestinal absorption of curcumin, and curcumin's micelle with phospholipid as well as bile salt was investigated. *In vitro* intestinal absorption of curcumin increased from 47% to 56% in case of micellar curcumin formulation.

In a study carried out by **Narayanan et al. (2009)** in which a combination of resveratrol and curcumin was used, a significant decrease of prostatic adenocarcinoma in PTEN knockout mice and *in vitro* studies on PTEN-CaP8 cancer cells revealed that resveratrol in combination with curcumin inhibited cell growth and induced apoptosis.

To increase the effectiveness of resveratrol, drug delivery system like nanocapsules can be used to target drugs at specific sites within the body in the field of cancer biology.

Polymeric Drug Conjugates with Ligands

Ligands are one of the biomarkers that can be used to differentiate between cancer tissue and normal tissue. Attaching ligands to the surface of nanoparticles can help recognize and bind selectively to the receptors that are expressed on tumour cells. This technique will help deliver high doses of anticancer drug directed specifically to the tumour cells sparing the normal cells, thus decreasing the side effects associated with the drug. Inclusion of a targeting antibody or ligand into polymer-drug conjugates has been the most suggested approach to encounter these limitations.

Dendrimers

In a study, PAMAM encapsulated curcumin and free curcumin were tested on T47D breast cancer cell line for their comparative antiproliferative effect. Using TRAP assay, telomerase activity was studied after 24 hrs of incubation. Inhibitory effect was found to be increased in telomerase activity. No cytotoxicity on cancer cells was found when treated with PAMAM dendrimers encapsulating curcumin. It also showed increase in antiproliferative activity of curcumin. (General representation of dendrimer is as shown in.

Nanoemulsions

It was observed that nanoemulsion-based delivery systems by encapsulation of polyphenols improved their water dispersibility and protected them from degradation as well as preserving the antioxidant activity. It was also observed that stability of resveratrol was improved when resveratrol (0.01% wt) was encapsulated in peanut oil-based nanoemulsions as shown by the significant reduction of the chemical degradation of *trans*-resveratrol to *Cis*-resveratrol. **Donsi et al. (2011)** said that as far as curcumin (0.1% wt) is concerned, it was encapsulated in solid lipid nanoemulsions that trapped the compound in a solid matrix, which lead to improved solubility in aqueous systems and to avoiding the recrystallization and settling of the bioactive compound over time.

Nanogels

Nanogels are crosslinked polymer network ranging size between 10 and 200 nm. *In vitro* studies were performed on breast cancer, melanoma, and pancreatic cell lines. Cell lines were treated by nanoparticles conjugated curcumin formulation. **Mangalathillam et al. (2012)** proved that nanocurcumin increased stability of curcumin, enhanced fluorescence effects, developed bioavailability, improved anticancer effects, got better controlled release, prolonged half-life, and enhanced treatment of melanoma.

Conclusions

In conclusion, TNBC is a difficult and complex disease entity that is both confusing and frustrating for researchers, physicians and patients. To date there are multiple approaches attempting to improve care of triple negative breast cancer patients, including DNA damaging agents like platinum's, and PARP inhibitors; however, none have been as clinically successful as anticipated and more targeted therapies need to be developed and explored. The Wnt/b-Catenin, NOTCH and Hedgehog signaling pathways are being considered as novel therapeutic targets for TNBC. To deliver optimizing therapies for most patients with TNBC, a comprehensive classification is necessary based on genomic data. This type of classifier will offer opportunities for both subtyping and subtype-guided therapy in patients with TNBC. In the near future, the subtypes of TNBC would be easily identified that, in conjunction with clinically available class, will help advance the management of women with TNBC.

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